Acta Agronomica Hungarica

An International Multidisciplinary Journal in Agricultural Science

VOLUME 53, NUMBER 1, 2005

Akadémiai Kiadó, Budapest

MEMBER OF WOLTERS KLUWER GROUP

ACTA AGRONOMICA HUNG. AAHUEX 53 (1) 1–118 (2005) HU ISSN 0238-0161

ACTA AGRONOMICA HUNGARICA

A QUARTERLY OF THE HUNGARIAN ACADEMY OF SCIENCES

Acta Agronomica Hungarica publishes papers in English on agronomical subjects, mostly on basic research

Acta Agronomica Hungarica is published in yearly volumes of four issues by

AKADÉMIAI KIADÓ, Budapest, Hungary

Acta Agronomica Hungarica is abstracted/indexed in Biological Abstracts, BIOSIS Previews, CAB Abstracts, Chemical Abstracts, GEOBASE, Global Healt, Index Veterinarius, International Bibliogrphics IBZ and IBR

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Acta Agronomica Hungarica
Agricultural Research Institute of the
Hungarian Academy of Sciences
H-2462 Martonvásár, Hungary
Phone: (36-22) 569-521 Fax: (36-22) 460-213
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The Agricultural Research Institute of the Hungarian Academy of Sciences contributes financially to the publication of *Acta Agronomica Hungarica*.

AAgr 53 (2005) 1

ACTA AGRONOMICA HUNGARICA

AN INTERNATIONAL MULTIDISCIPLINARY JOURNAL IN AGRICULTURAL SCIENCE

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AKADÉMIAI KIADÓ, BUDAPEST

Editorial

It is now just over ten years since the Editorial Office of *Acta Agronomica Hungarica* returned to the Agricultural Research Institute of the Hungarian Academy of Sciences. Throughout this period the journal was edited by József Sutka, whose dedication and attention to detail enabled it to appear regularly and, with the help of Akadémiai Kiadó, in a form meeting international standards. On the occasion of Professor Sutka's retirement the members of the Editorial Board join our readers in expressing our appreciation for his efforts, and assure him that his work will be continued, our aim being to maintain the journal as a forum for scientists conducting basic and applied research in agriculture, with the emphasis on *investigations carried out on cultivated plants*.

In order to further raise the standard of the journal certain changes will be made. Over the next few years it is planned to publish a number of *thematic issues* reporting on the work of research consortiums set up within the framework of Hungarian and international cooperation. The first of these will be the first two issues of Volume 53, which will contain mostly papers reporting the results achieved by the "Maize Consortium" project funded by a grant from the Ministry of Education (NKFP No. 4/008/2002). It is hoped to make *Acta Agronomica Hungarica* a forum for the publication of the papers and posters presented at international conferences held in Hungary on fields related to the journal's profile. The backing and financial support of the Agricultural Section of the Hungarian Academy of Sciences and the expertise of Akadémiai Kiadó will ensure that these aims can be achieved, thus allowing us to improve the professional standard of the journal and increase the number of readers, both at home and abroad.

Martonvásár, June 2005

EMIL PÁLDI
Editor

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*Papers reporting research carried out as part of the Maize Consortium Project.

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RESULTS OF COMPARATIVE FARM TRIALS ON NEW HUNGARIAN-BRED MAIZE HYBRIDS AND RECOMMENDATIONS TO FARMERS

J. NAGY[1] and L. HONTI[2]

[1]CENTRE OF AGRICULTURAL SCIENCES, UNIVERSITY OF DEBRECEN, DEBRECEN, HUNGARY
[2]KITE CO. LTD., NÁDUDVAR, HUNGARY

Received: 17 March, 2005; accepted: 30 May, 2005

Reliable small-plot experiments play an important role in determining the value of hybrids, but the results of large-plot experiments, which give a better reflection of conditions and technologies, are needed to obtain reliable information on which hybrids can be produced most economically under specific cultivation conditions.

Within the framework of the National Research Development Programme, large-scale tests are underway on new Hungarian maize hybrids, to promote the selection, introduction and propagation of the most economical hybrids for specific regions, ecological conditions and technological standards.

The tests included hybrids Sze SC 271 (FAO 290), Dáma (FAO 300) and Mv 277 (FAO 320) from the very early maturity group, Sze SC 352 (FAO 340), Hunor (FAO 370) and Norma (FAO 380) from the early maturity group and Maraton (FAO 450) and Sze SC 463 R (FAO 490) from the medium maturity group, grown at 20 different locations in Hungary.

Each year the yield differences between the locations reflected the extreme distribution of precipitation during the growing season in different parts of the country. High amounts of precipitation were required in the growing season to reach the highest yields, but moderately high yields were achieved over a wide range of rainfall levels.

Key words: maize, Hungarian breeding, cultivation site, extension service

Introduction

According to economic forecasts a continuous increase in the demand for maize can be expected in the long term, both on European and world markets. Maize has played a decisive role in Hungarian crop production for several decades. The profitability of the maize sector can be ensured by improving the yield stability of maize hybrids.

Our aim is to include new genotypes in scientific experiments, which will produce information of great value for both science and practice. These have a continuous role in the development of plant production, in research and practice, in education and in the extension service. The breeding of new varieties and hybrids, their state registration and their introduction into mass production constantly require the inclusion of new genotypes in scientific experiments. Thus, it is important to develop hybrid-specific maize production technologies adapted to the cultivation site, the application of which will have a positive influence on the profitability of crops, while a reduction in the use of chemicals will minimize environmental impacts.

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Since the weather regulates the heat and moisture supplies of the growing site it has an effect on the transformation of elements in the soil, and on the growth, nutrient uptake and fertilizer utilization of plants (Cselőtei, 1978; Ángyán et al., 1982; Menyhért, 1985; Szász, 1988; Nagy, 1993). The majority of experiments indicate that in moderately dry years the fertilizer effect is medium or good. In the case of drought, the plants develop well in the first phase of plant growth, while in the second phase the high LAI and the increased water requirements lead to severe water deficiency, resulting in significant yield losses (Győrffy et al., 1965; Debreczeni and Debreczeniné, 1983). Berzsenyi (1993) used various biometrical methods to prove the decisive role of N fertilization and year on the yield of maize, based on over twenty years of experiments. A comparison of dry and wet years showed that the yield increment in different N treatments in wet years amounted to: N_0: 1.13; N_{80}: 2.14; N_{160}: 2.28 and N_{240}: 1.99 t/ha, while both the N fertilizer × hybrid and the year × N fertilizer interactions were significant. The amount of precipitation and the moisture reserves of the soil modify both the fertilizer requirement and the fertilizer effect. The effect of fertilization increases when the water supply approaches optimum, and decreases again if the water supply is excessive. The influence of soil characteristics depends primarily on soil fertility, the thickness of the topsoil and the water balance (Sarkadi, 1975; Ruzsányi, 1974). The forecrop may directly reduce or increase the water and nutrient resources of the soil and may have other effects (e.g. on the extent of weed cover) that modify the fertilizer effect (Győrffy and Berzsenyi, 1992). Optimal N supplies significantly contribute to the favourable formation of yield components (Bocz and Nagy, 1981). In the case of N deficiency, dry matter accumulation is lower in maize plants and the dynamics of dry matter accumulation is slow (Németh, 1978; Hanway and Russell, 1969).

Materials and methods

Within the framework of the National Research Development Programme, new Hungarian maize hybrids are being farm tested to promote the selection, introduction and popularization of the most economical hybrids for specific regions, ecological conditions and technological standards.

The tests included the hybrids Sze SC 271 (FAO 290), Dáma (FAO 300) and Mv 277 (FAO 320) from the very early maturity group, Sze SC 352 (FAO 340), Hunor (FAO 370) and Norma (FAO 380) from the early maturity group, and Maraton (FAO 450) and Sze SC 463 R (FAO 490) from the medium maturity group, grown at 20 different locations in Hungary under farm conditions and in comparison with other hybrids (Fig. 1).

The sowing, weed control and harvesting of the hybrids were carried out at the technological level of the farm in question. A mobile balance was provided by the KITE Co. to ensure precise weighing. Samples were taken from the top 25 cm of the soil to analyse the phosphorus, potassium and nitrogen balances. The samples were collected in the stubble after harvesting the forecrop.

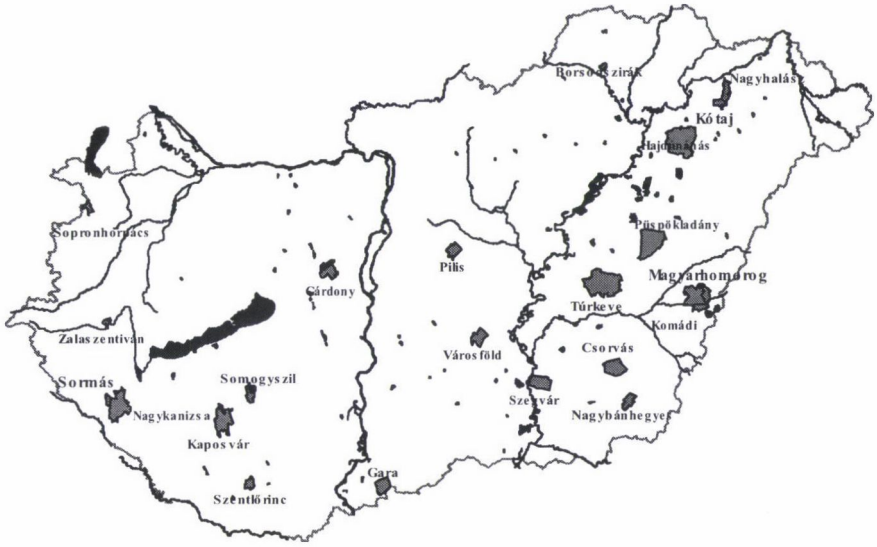

Fig. 1. Locations of farm tests on maize hybrids

The results were evaluated using the SPSS for Windows 11.0 statistical software package. Regression analysis was used to evaluate the data (Huzsvai, 2001). When fitting the functions the sums of squares was minimised. The goodness of fit was checked using the r-value and the size of the estimation error. The function parameters were tested with the t-probe, at the 5% significance level.

Results and discussion

The quantity and distribution of rainfall differed greatly over locations and years. Every year the yield differences between the locations provided a good illustration of the distribution of precipitation during the growing season in different parts of the country.

The year 2004 was favourable for maize production, especially as regards precipitation, while the opposite was true of 2002, and 2003 was intermediate between these two years. The correlation between precipitation during the growing season and the yield of the tested maize hybrids is illustrated by the location means found in Table 1. High quantities of precipitation are required in the growing season if high yields are to be obtained, while moderate or low yields may be achieved with varying rainfall levels. A favourable distribution of precipitation improved yield stability, as shown by the coefficient of variation for the yields at various locations.

Table 1
Correlation between rainfall (mm) during the growing season and the yields (t/ha) of tested maize hybrids (2002–2004)

Year		Transdanubia		Great Plain		Average	
		t/ha	C.V.	t/ha	C.V.	t/ha	C.V.
2002	Yield	8.2	2.6	5.9	2.1	7.1	2.3
	Rainfall in growing season	264	24	300	50	281	40
2003	Yield	6.1	1.4	6.4	2.0	6.3	1.7
	Rainfall in growing season	204	28	202	43	203	37
2004	Yield	10.1	1.1	9.1	0.6	9.6	1.0
	Rainfall in growing season	378	22	356	20	367	24

C.V. = coefficient of variation between locations

Table 2
Humus content (%) and fertilizer application (active ingredients kg/ha) at locations in Transdanubia and the Great Plain in Hungary

Locations in Hungary	Humus content	Average fertilizer application		
		N	P	K
Transdanubia	2.2	120	40	60
Great Plain	3.0	100	30	25

The effect of precipitation during the growing season on the grain yield was calculated using multivariate linear regression. The average grain yield response to 1 mm of precipitation was 13 kg/ha in 2002, 46 kg/ha in 2003 and 26 kg/ha in 2004. The tested hybrids gave higher grain yield averages in Transdanubia than on the Great Plain. This can be attributed not only to the better distribution of rainfall (lower C.V.) but also to the higher technological standard.

An evaluation of the data in Table 2 showed that, averaged over many years, the humus content (%) was lower in Transdanubia than on the Great Plain. However, at the farm locations the amount and nutrient ratio of fertilizer applied was more favourable in Transdanubia than on the Great Plain.

The absolute and relative yields of the tested maize hybrids compared to the checks can be found in Table 3. The new Hungarian maize hybrids are worthy of a place in maize production in Hungary. Hungarian farmers using medium or low technological inputs can choose with great safety from this variety collection. In years with weather unfavourable for maize production, which are becoming increasingly frequent, or for farmers using low or medium input technologies, the choice of Mv 277 (107% in 2002, 103% in 2003), Dama (104% in 2002, 98% in 2003) or Maraton (100% in 2002, 100% in 2003) could lead to surplus or equal yields compared with other hybrids. In good years for maize these hybrids lose their advantage over other hybrids (Fig. 2).

Table 3
Yield of maize hybrids in 2002–2004 (20 locations a year)

Hybrid	Year					
	2002		2003		2004	
	t/ha	%	t/ha	%	t/ha	%
Mv 277	7.2	107	6.0	103	9.6	102
Sze Sc 271	–	–	5.7	98	8.8	94
Dama	7.0	104	5.7	98	–	–
Average for the FAO 280 to FAO 320 group	6.7	100.0	5.8	100.0	9.4	100.0
Sze Sc 352	–	–	6.4	102	10.5	108
Hunor	7.6	106	6.9	110	10.2	105
Norma	6.7	93	6.2	98	–	–
Ella	6.9	96	5.9	94	–	–
Average for the FAO 321 to FAO 399 group	7.2	100.0	6.3	100.0	9.7	100.0
Sze Sc 463 R	7.3	91	6.2	102	9.4	99
Maraton	8.0	100	6.1	100	8.9	94
Average for the FAO 400 to FAO 499 group	8.0	100.0	6.1	100.0	9.5	100.0

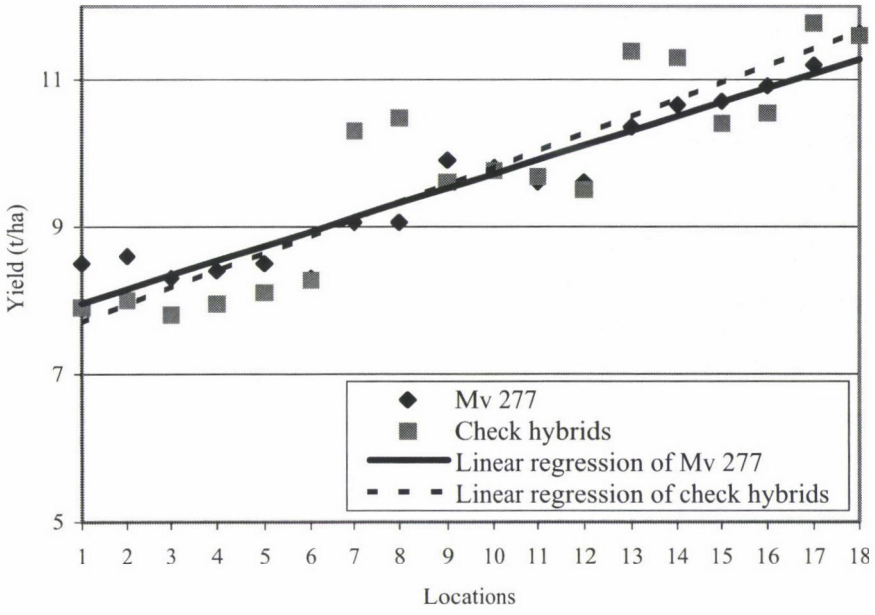

Fig. 2. Yield of Mv 277 and check hybrids in 2004

Hunor performed well both in different years and at various technological levels (106% in 2002, 110% in 2003, 105% in 2004). The hybrid Sze SC 352 is best able to achieve its high yield potential in good years with efficient production technologies. The choice of this hybrid will improve yield stability and reduce costs, as the seed is cheaper than that of other hybrids (Fig. 3). The hybrid Norma has average yield combined with good adaptability, making it ideal for farmers working under poorer production conditions. For farms with better conditions (rainfall supplies, heat sum, technology) hybrids with a longer vegetation period are recommended, which require more fertilizer and water (for example: Maraton, Sze SC 463 R).

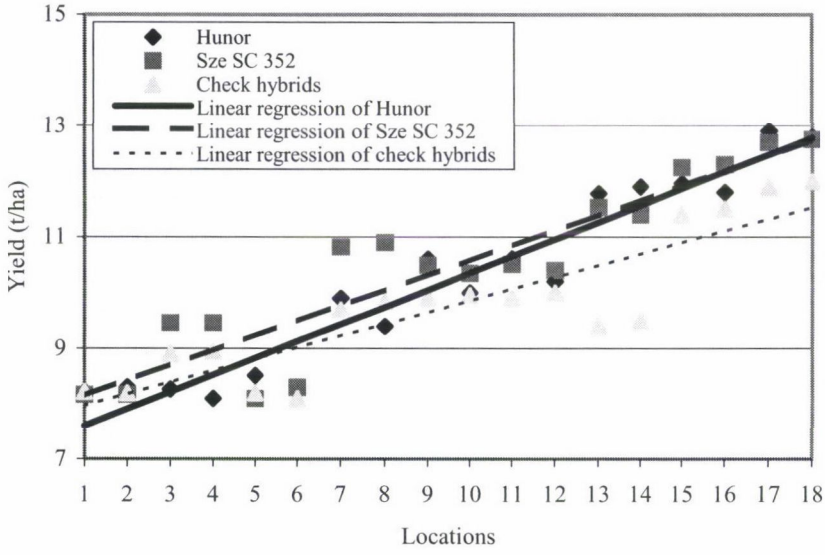

Fig. 3. Yield of Hunor, Sze SC 352 and check hybrids in 2004

Acknowledgements

This research was carried out with the support of grant No. OM 4/0008/2002.

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CHANGES IN THE QUALITY OF MAIZE HYBRIDS IN VARIOUS AGRICULTURAL MANAGEMENT SYSTEMS

Z. GYŐRI, P. SIPOS and Á. TÓTH

DEPARTMENT OF FOOD SCIENCE AND QUALITY ASSURANCE, CENTRE OF AGRICULTURAL SCIENCES, UNIVERSITY OF DEBRECEN, DEBRECEN, HUNGARY

Received: 31 March, 2005; accepted: 25 April, 2005

Changes in the chemical contents of the kernels of maize hybrids were analysed in three consecutive years (one dry, one arid and one wet) in samples originating from irrigation and fertilisation experiments. The results indicated that while irrigation and wet years had a protein-decreasing effect, the water supply did not influence the starch and oil contents. Under the influence of fertilization, the calcium and zinc contents of maize kernels decreased to varying degrees depending on the hybrid, while the sulphur contents changed parallel to the protein contents. Based on these data and on the use of more modern measuring equipment, tables giving recommendations for chemical contents should be revised.

Key words: maize hybrids, quality, external factors, Zn, S, Ca content, protein, oil, starch

Introduction

The yields of maize, the crop grown on the largest area in Hungary apart from wheat, are influenced by several factors, as indicated by numerous authors. Apart from fluctuations in the yields, these authors also note the importance of testing various factors influencing kernel quality, the importance of which is continuously increasing. The analysis of maize grain yields is extremely important for both animal feeding and human nutrition, with the latter coming more and more to the foreground. At the same time, the detection limits are getting lower and lower, the precision of measurement is increasing, and the range of parameters tested is widening.

Identical trends can be observed in the nutrient responses of hybrids, even in the case of different genetic backgrounds: increasing ratios of nitrogen will increase the nitrogen content of the yield significantly and in an almost linear way (Balla, 1960; Latkovics, 1961; Lásztity, 1975; Sarkadi, 1975). On the other hand, a slightly negative linear correlation has been demonstrated between average maize yields and their crude protein contents (Bálint, 1977). Irrigation decreases the nitrogen content (Debreczeni, 1965; Ruzsányi, 1977), so it has to be supplemented with the intensive application of artificial fertilizers if higher protein contents are to be achieved (Debreczeni, 1979).

Allen (1979) and Salunkhe et al. (1985) gave average values of 1400 mg/kg for the sulphur contents of maize kernels, which showed a sharp rise as the result of phosphorus, calcium and sulphur fertilization (Tölgyesi, 1991).

0238–0161/$ 20.00

While FAO (1992) data gave 483 ± 123 mg/kg as the average calcium content of maize kernels, Kádár et al. (2000) reported much lower concentrations of calcium (99–138 mg/kg) and found that increasing rates of potassium fertilizers decreased the calcium content due to antagonism. The parallel application of P and K fertilizers was reported to decrease the Zn concentration as well as that of calcium (Lásztity et al., 1985), while the application of high rates of potassium was found to decrease the Zn content (Prohánszka and Cserni, 1969; Ragab, 1980).

Materials and methods

The kernels analysed in the experiments were grown at the Experimental Station of the Centre for Agricultural Sciences of the University of Debrecen in Látókép in the years 2002–2004. The soil of the experimental farm is a calcareous chernozem formed on lowland loess. It has average N and P supplies and a high K supply (humus content = 2.8–3%, total N = 0.14–0.18%, AL-P_2O_5 130–200 mg/kg, AL-K_2O = 240–280 mg/kg, pH_{KCl} 6.2). The depth of the humus layer is 70–90 cm.

The years 2002 and 2003 were very dry. During the warm months of spring and summer the amount of precipitation was only 150–160 mm, which greatly hindered the success of maize production. The situation in 2003 was aggravated by the lack of precipitation in the preceding year. In 2004 a total of 320 mm of precipitation was recorded during the same period, the distribution of which was also favourable for the development of maize.

Three factors: irrigation, hybrid and nutrient supply, were studied in the experiment, which was set up in four replications. The hybrids tested were Maraton, Ella and Debreceni 377 in 2002, Debreceni 377, Mv 370, Norma and Katinka in 2003, and Hunor, Mv 277, Debreceni 377 and Norma in 2004. The fertilizer ratios applied are shown in Table 1. Quality tests were carried out on kernel samples from the control and from treatments 2 and 4.

The laboratory tests were conducted in the Regional Agricultural Equipment Centre of the Centre for Agricultural Sciences of the University of Debrecen. The analyses were based on the following standards: protein content MSZ 6830-4:1981, starch content MSZ 6830-18:1988 and crude oil content MSZ 6830-6:1984. The element contents were determined with OPTIMA 3300 DV (Perkin-Elmer Ltd., Wellesley, USA) ICP-OES equipment using the method of Kovács et al. (1996; 1998). This instrument is capable of determining the contents of several elements simultaneously, but the present paper concentrates on the sulphur, calcium and zinc contents.

The effects of years and agronomical factors were examined by analysis of variance. Data processing was carried out using the statistical program package SPSS 11.01.

Table 1
Fertilizer treatment applied in the experiment (kg/ha)

Treatment	N	P_2O_5	K_2O	Total
0	–	–	–	–
1	30	23	27	80
2	60	46	54	160
3	90	69	81	240
4	120	92	108	320
5	150	115	135	400

Results

An analysis of the protein contents of the hybrids revealed that on average the protein contents were higher in 2002 and 2003 than in the wet cropping year of 2004 (annual averages were 8.97, 8.72 and 8.22%). In 2004 irrigation had a moderate effect on the protein concentration, while in 2002 and 2003 the protein contents of the hybrids decreased by about 0.7% as a result of irrigation. An increase in the fertilizer rates clearly resulted in increased protein contents (Fig. 1).

The starch and oil contents were analysed under droughty conditions in 2003 and it was found that under these cropping conditions irrigation had no modifying effect on these parameters, while fertilizer application led to a decrease in their values in certain cases (Figs. 2 and 3).

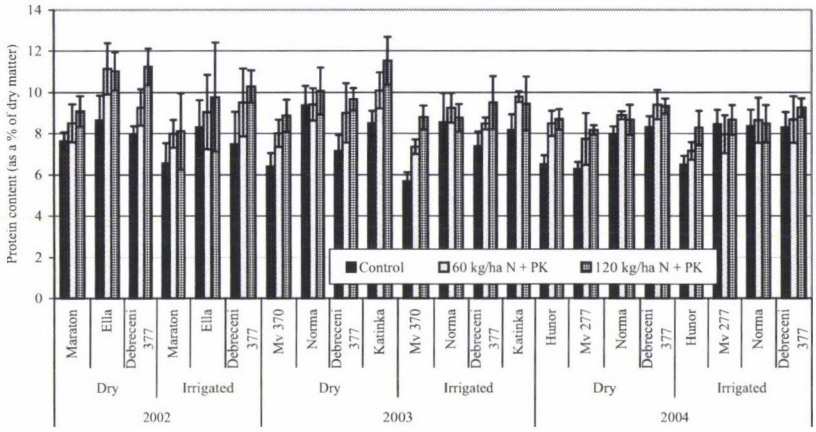

Fig. 1. Effects of agricultural management, hybrids and years on the protein content of maize hybrids (Látókép, 2002–2004)

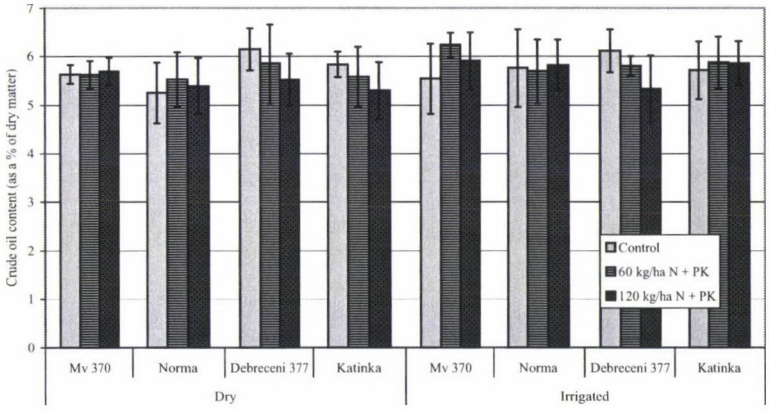

Fig. 2. Effects of hybrids and agricultural management on the crude oil content of maize hybrids (Látókép, 2003)

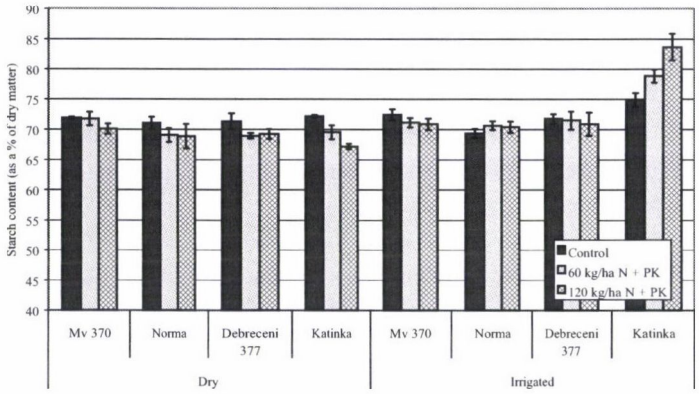

Fig. 3. Effects of hybrids and agricultural management on the starch contents of maize hybrids (Látókép, 2003)

An analysis of the sulphur content in the hybrids revealed that irrigation and the year had a joint effect on the sulphur concentration (Fig. 4). In the wet year of 2004 kernels from the non-irrigated plots had a S content in the region of 950–1000 mg/kg, while irrigated plots contained 900–950 mg/kg. The distribution curve for the dry year of 2002 exhibited two peaks resulting from fertilizer application, while the water supply had no significant effect on the S content. The most marked difference resulting from varying levels of water supply was observed in the droughty year of 2003, when the sulphur contents of hybrids grown under irrigated conditions were 150 mg/kg less on average than those of plants grown under non-irrigated conditions. Based on the findings for the period analysed it can be stated that in moderately dry years the nutrient supply has a significant positive effect on the sulphur contents of maize kernels, while in wet and dry years the negative influence of the water supply is dominant.

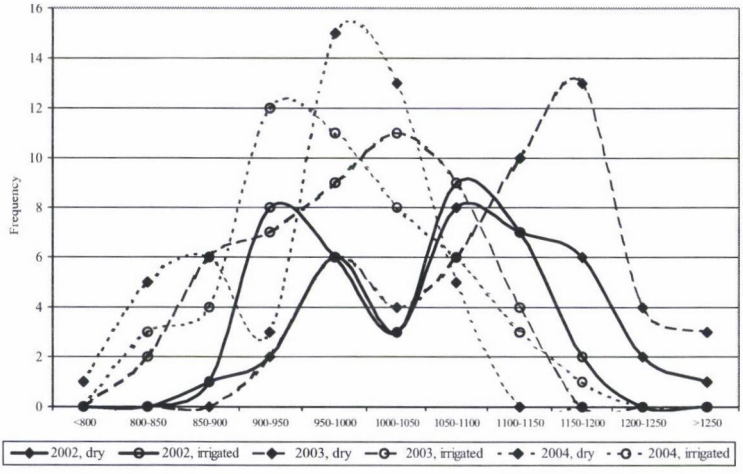

Fig. 4. Distribution of sulphur contents in the years of the experiment (Látókép)

When analysing the Ca contents of the hybrids it was found that in the case of all the samples and for hybrids Debreceni 377 and Norma, both of which were grown in two years, the year, the hybrid and fertilizer application significantly influenced the Ca contents of the kernels. The highest values were found for the hybrid Norma and in 2003. Apart from a few exceptional cases the application of increasing fertiliser rates resulted in a decrease. Values contradicting this trend were found on non-irrigated plots in the dry years and on moderately wet plots in 2004 (Fig. 5). The Ca contents of the kernels ranged between 36.3 and 106.8 mg/kg. These findings show that the calcium contents of kernels are significantly less than those determined earlier (FAO, 1992).

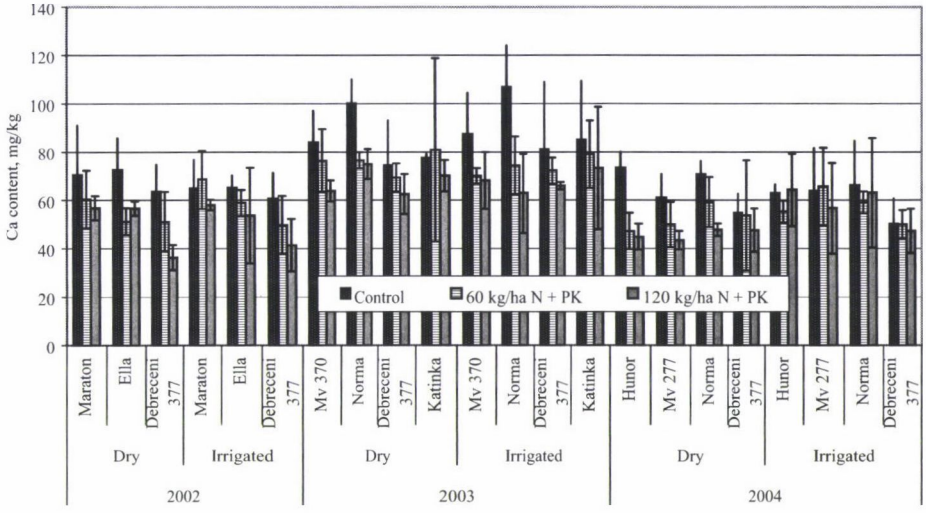

Fig. 5. Effects of hybrids, years and agricultural management on the Ca contents of maize hybrids (Látókép, 2002–2004)

Discussion

An analysis of the chemical contents of kernels of maize hybrids grown under various weather conditions indicated that wet years resulted in a decrease in protein contents similar to that observed when irrigation was applied. In 2002 and 2003 the protein contents decreased as a result of irrigation by 0.83 and 0.57%, respectively, while the protein content of the grain was 0.75 and 0.50% lower in 2004 than in 2002 and 2003, respectively. The water supply did not influence the oil or starch contents, though it had a decreasing effect on these parameters when higher fertilizer rates were applied. In a similar way to the nitrogen content, the sulphur content of the kernels in moderately dry years was typically increased by fertilizer application, while in wet and dry years irrigation had a decreasing effect. Calcium contents, which were considerably lower than

those cited in the literature (between 36 and 107 mg/kg, typically 60–80 mg/kg), showed a decrease as a result of increasing fertilizer rates. In order to maintain adequate levels of zinc in maize kernels, special attention must be paid to the individual properties of the hybrids, and the interaction between phosphorus and zinc must be considered when determining the rate of phosphorus. In the present experiment hybrids Mv 370 and Ella showed the greatest sensitivity to phosphorus-zinc antagonism, the influence of which may be modified by Zn application at an early phenological stage.

Acknowledgements

This study was supported by grants Nos. OM-136/2002 and OMFB-00207/2003.

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Acta Agronomica Hungarica, 53(1), pp. 17–29 (2005)

NEW RESULTS IN THE HYBRID-SPECIFIC WEED CONTROL OF MAIZE (*ZEA MAYS* L.)

P. PEPÓ

DEPARTMENT OF PLANT PRODUCTION AND APPLIED ECOLOGY, FACULTY OF AGRONOMY, CENTRE OF AGRICULTURAL SCIENCES, UNIVERSITY OF DEBRECEN, DEBRECEN, HUNGARY

Received: 17 March, 2005; accepted: 22 April, 2005

The effects of herbicides containing different active agents and applied at different times (early, normal and late post-emergence) were studied on a chernozem soil in the Hajdúság region of E. Hungary in stands of different hybrid maize genotypes. The results proved that the extent of weed cover was equally influenced by the forecrop and the year. In a drier year with wheat as the forecrop, moderate weed infestation was recorded, while with maize as the forecrop and more favourable water supplies, infestation was more severe.

The hybrid did not have a significant effect on the extent of weed cover. Little (2–6%) or no visible phytotoxicity was observed in the maize hybrids as a consequence of post-emergence herbicide treatments. The yield-enhancing effects of the various herbicides changed in proportion to the weed cover (in comparison to the untreated weedy control). In the case of low weed cover (in 2002 the weedy control was 7.6–8.4%) there were yield increases of 0–500 kg ha^{-1}, which increased to 700–2700 kg ha^{-1} in the case of moderate weed cover (in 2003 the weedy control was 27.4–35.1%) and to 3200–4200 kg ha^{-1} when weed infestation was severe (in 2004 the weedy control was 52.3–57.4%). The study proved that maize hybrids with different genotypes demonstrated general and specific types of herbicide sensitivity (active agent, timing of application).

Key words: maize, herbicides, hybrids, weed control, yield

Introduction

Cereals, especially wheat and maize, play an important role in Hungarian crop production. The national maize yields have undergone a significant transformation in recent decades. Inbred hybrids, which were introduced very rapidly at the beginning of the 1960s, ensured an adequate genetic basis for the wide use of inputs of industrial origin. From the 1960s the use of fertilizers dynamically increased, chemical herbicides spread widely, and modern machines and appliances were used. The role of agronomic factors in the production technology of maize was defined by Győrffy (1976) as follows: fertilization 27%, hybrid 26%, cultivation 24%, plant density 20%, and tillage 3%.

In the 1980s the international competitiveness of Hungarian maize production was well characterized by a national yield of about 6.0 t ha^{-1} and the fact that the fluctuation in national yields was very moderate (10–20%). From the beginning of the 1990s, due to the widely known financial and economic difficulties, the yield of maize decreased by 0.5–2.0 t ha^{-1}, depending on the year, but because of the low level of industrial inputs the fluctuation significantly increased (30–50% in the case of the national yield).

0238–0161/$ 20.00

One of the important elements in maize production is the use of herbicides. Modern weed control should be based on the principle of integration (Széll et al., 1985; Chui et al. 1997; Berzsenyi, 2000). The significance of early and normal post-emergence treatment has increased considerably in cases where the composition of the weed cover is known. The effect and efficiency of post-emergence treatments depends on environmental factors (Tapia et al., 1997) and on the sensitivity of the hybrids (Bónis et al., 2000; Hart and Wax, 1999). In recent years the number of registered grain maize and silage maize hybrids has increased greatly to almost 400. These genotypes differ significantly in their agronomic traits, thus showing a hybrid-specific response. It is also important to emphasize that all herbicide treatments have some stress effect on the growth of maize, which is manifested in the agronomic and phenometric characteristics and also in the yield.

Bujtás and Németh (1996) proved that the phytotoxicity caused by herbicides depended on the soil type. Their results indicated that the strongest toxicity was found in acidic sandy soil, while the effects were far less severe in acidic but heavily textured soil, while in a calcareous chernozem soil normal field application rates led to a slight increase in growth. Solymosi (1984) stated that tolerance to herbicides was a more general phenomenon than resistance. Isoxaflutol (Merlin), which inhibits chlorophyll synthesis, is useful for controlling annual broad-leaf weeds and some grasses. The effective dosage rate is dependent on soil type and the number of weeds. To improve the efficacy of isoxaflutol against grasses it is necessary to add a graminicide. Stefanovic et al. (2000) examined the damage rating and phytotoxicity of a post-emergence herbicide (2,4-D) on three maize groups (FAO 300–400, FAO 500, FAO 600–700) including 51 maize hybrids in different growth stages (4–5 and 7–10-leaf stage). The highest general damage rating was observed in hybrids from the early maturity group (FAO 300–400). Phytotoxicity was influenced by the meteorological conditions during herbicide application and by the timing of application (delayed application caused higher phytotoxicity).

Materials and methods

The small-plot experiments were performed at the Látókép Experimental Station of the Department of Crop Production and Applied Ecology, University of Debrecen, on calcareous chernozem soil. The soil of the experiment was nearly neutral (pH_{KCl} 6.46), with average phosphorus and potassium contents (AL-soluble P_2O_5 133 mg/kg, AL-soluble K_2O 240 mg/kg) according to soil analysis. The soil had favourable water metabolism and water-holding characteristics, was in good condition, and was ideal for maize production.

The experiments were carried out between 2002 and 2004 with optimum crop management as regards soil tillage, fertilization, planting and harvest. The forecrops were winter wheat in 2002, and grain maize in 2003 and 2004.

In all years the following hybrids were tested: De 377 SC, Veronika, Borbála, Gazda, Maraton and Norma.

The following herbicide treatments were applied each year:

1. Weedy control
2. Mechanically hoed control
3.* Dual Gold 1.0 kg ha^{-1} + Callisto 0.3 l ha^{-1} + Gesaprim 1.0 kg ha^{-1} (early post-emergence)
4. Merlin 140 g ha^{-1} + Dezormon 1.0 l ha^{-1} (early post-emergence)
5.* Dual Gold 1.6 l ha^{-1} + Callisto 0.3 l ha^{-1} + Gesaprim 1.0 kg ha^{-1} (normal post-emergence)
6. Merlin 140 g ha^{-1} + Dezormon 1.0 l ha^{-1} (normal post-emergence)
7. Motivel 1.0 l ha^{-1} + Cambio 3.0 l ha^{-1} (normal post-emergence)
8. Titus 25 DF 40 g ha^{-1} + Callisto 0.25 l ha^{-1} + Trend 0.1% (normal post-emergence)
9. Motivel 10 l ha^{-1} + Cambio 3.0 l ha^{-1} (late post-emergence)
10. Titus 25 DF 40 g ha^{-1} + Callisto 0.25 l ha^{-1} + Trend 0.1% (normal post-emergence)

The treatments were performed in the following growth stages of maize:

Mechanical hoeing: twice during the vegetation period (between mid-May and early June)

Early post-emergence treatment: in the 2–3-leaf stage

Normal post-emergence treatments: in the 5–7-leaf stage

Late post-emergence treatments: in the 9–10-leaf stage.

The most important meteorological data during the vegetation period of maize can be found in Tables 1 and 2.

The meteorological conditions in the 2002 and 2004 seasons were very favourable for the vegetative growth and yield formation of maize. In 2003 the water supply was less favourable and the temperature higher compared with the other two years. The lower yields (generally 1–2 t ha^{-1} less) proved that the weather conditions were slightly unfavourable in 2003.

Data were collected on growth dynamics, agronomic traits, phyto-sanitary data, weed dynamics, phytotoxicity, yield components, grain water content at harvest and yields.

Table 1

Precipitation (mm) during the vegetation period of maize (Debrecen, 2002–2004)

Years	April	May	June	July	August	September
2002	16.0	11.8	61.5	46.6	51.7	64.9
2003	13.7	54.4	22.2	84.5	1.2	46.0
2004	40.0	17.0	61.7	142.2	50.2	31.3
30-year average	42.4	58.8	79.5	65.7	60.7	38.0

Table 2

Temperature (°C) during the vegetation period of maize (Debrecen, 2002–2004)

Years	April	May	June	July	August	September
2002	9.8	17.5	19.0	21.8	19.4	13.8
2003	9.2	19.1	21.3	21.3	22.4	14.4
2004	11.4	14.8	19.3	21.1	20.4	15.3
30-year average	10.7	15.8	18.7	20.3	19.6	15.8

*These treatments were applied only in 2003 and 2004.

Results

Considerable changes have taken place in the weed control of maize over the past decade, which is due partly to the appearance of new weed-killers and changes in the environment (mainly weather conditions), but also to changes in the composition of the weed population, farm ownership structure and the level of mechanisation. The proportion of pre-sowing treatments, which were widely used earlier, but which require incorporation into the soil, decreased considerably, together with the percentage of pre-emergence treatments. The proportion of post-emergence treatments rose considerably, partly due to changes in the weed composition (the extensive appearance of late emerging and resistant weeds) and also as the result of cost-efficient and environment-friendly treatments specifically designed for the weed flora. It should be noted, however, that post-emergence treatments also have their dangers and limitations. The dangers are posed by the sensitivity of various maize hybrids to herbicides and the limitations are represented by the fact that the treatments have to be carried out at specific stages of phenological development. Hybrids with different genotypes show different patterns of sensitivity to post-emergence weed-killers, which often manifests itself in minimal levels of phytotoxicity but considerable reductions in yields. In the case of post-emergence treatments, delaying treatment after the optimum date (e.g. a normal post-emergence treatment instead of an early one, or a late post-emergent treatment instead of a normally timed one) may result in phytotoxicity and in yield losses. The present experiments, conducted over several years, were designed to test the responses of different maize hybrids to optimal and late post-emergence weed-control treatments using various herbicides. Phytotoxicity, the efficiency of weed control (% of weed cover) and the effect on yields were also studied.

The weed-killing effects of the herbicide treatments and the percentages of weed cover are shown in Tables 3 and 4 for the different years and hybrids. The results demonstrated that the forecrop and the growing season greatly influenced the extent of weed infestation even within the same treatment, while the hybrids did not have a significant effect on weed cover. The forecrops were wheat in 2002 and maize in 2003 and 2004. The data for the natural weed cover are represented by the values for Treatment 1 (weedy control). Following wheat as forecrop the rate of weediness was between 7.6 and 8.4%, while after maize forecrops the rates of weediness were 27.4 to 35.1% in 2003 and 52.3 to 57.4% in 2004, depending on the hybrid. The effect of the year is illustrated by the results for 2003 and 2004, when maize was the forecrop in both cases. In the drier year of 2003 the extent of weed infestation in Treatment 1 was about half (27.4–35.1%) of that in the wetter year of 2004 (52.3–57.4%). Mechanical weed control (Treatment 2) reduced the weed cover to a moderate extent. On the average of 2002 and 2003 the weed infestation of 29.7–33.4% in the weedy control treatments decreased to 18.7–19.9% as a result of two between-row

cultivator treatments. Averaged over all three years, the extent of weed infestation decreased considerably as a result of herbicide applications in practically all the treatments. Herbicides applied at the correct time (Treatments 3, 4, 5, 6, 7 and 8) proved to be the most efficient, while the weed-killing effects of late treatments (Treatments 9 and 10) were more moderate (due to the larger leaf area of the more developed maize population). Depending on the hybrids the extent of weed infestation, averaged over three years, was 6.2–7.7% in Treatment 3, 4.5–6.3% in Treatment 4, 3.7–4.7% in Treatment 5, 3.3–3.9% in Treatment 6, 1.9–2.5% in Treatment 7, 4.3–5.0% in Treatment 8, 10.3–10.7% in Treatment 9, and 10.9–11.8% in Treatment 10.

Table 3
Effect of herbicides on the weediness (%) of maize (Debrecen, 2002–2004)

Hybrids and herbicide treatments	24 Sep. 2002	15 Sep. 2003	1 Oct. 2004	Average
De 377 SC				
1. Weedy control	7.9	31.2	54.2	31.1
2. Hoed control	4.3	16.4	37.2	19.3
3. Dual Gold + Callisto + Gesaprim (early post)		10.7	4.6	7.7
4. Merlin + Dezormon (early post)	1.7	13.0	4.1	6.3
5. Dual Gold + Callisto + Gesaprim (normal post)		3.1	6.3	4.7
6. Merlin + Dezormon (normal post)	1.4	2.3	7.9	3.9
7. Motivel + Cambio (normal post)	1.2	2.1	3.1	2.1
8. Titus 25 DF + Callisto + Trend (normal post)	1.2	5.4	8.1	4.9
9. Motivel + Cambio (late post)	2.4	12.7	16.3	10.5
10. Titus 25 DF + Callisto + Trend (late post)	2.1	15.3	17.9	11.8
Veronika				
1. Weedy control	7.6	27.4	54.2	29.7
2. Hoed control	5.0	14.7	36.4	18.7
3. Dual Gold + Callisto + Gesaprim (early post)		10.3	4.3	7.3
4. Merlin + Dezormon (early post)	1.6	12.1	3.4	5.7
5. Dual Gold + Callisto + Gesaprim (normal post)		2.9	5.8	4.4
6. Merlin + Dezormon (normal post)	1.7	2.4	6.9	3.7
7. Motivel + Cambio (normal post)	1.1	2.5	3.1	2.2
8. Titus 25 DF + Callisto + Trend (normal post)	1.2	4.7	6.9	4.3
9. Motivel + Cambio (late post)	2.4	13.1	16.3	10.6
10. Titus 25 DF + Callisto + Trend (late post)	2.1	14.2	17.8	11.4
Borbála				
1. Weedy control	8.2	34.1	56.3	32.9
2. Hoed control	4.7	16.7	38.1	19.8
3. Dual Gold + Callisto + Gesaprim (early post)		9.3	4.8	7.1
4. Merlin + Dezormon (early post)	1.9	8.5	3.9	4.8
5. Dual Gold + Callisto + Gesaprim (normal post)		2.4	6.8	4.6
6. Merlin + Dezormon (normal post)	1.4	2.0	7.2	3.5
7. Motivel + Cambio (normal post)	1.2	1.7	2.9	1.9
8. Titus 25 DF + Callisto + Trend (normal post)	1.4	5.7	7.8	5.0
9. Motivel + Cambio (late post)	2.2	14.5	15.3	10.7
10. Titus 25 DF + Callisto + Trend (late post)	2.1	12.5	19.4	11.3

Table 4
Effect of herbicides on the weediness (%) of maize (Debrecen, 2002–2004)

Hybrids and herbicide treatments	24 Sep. 2002	15 Sep. 2003	1 Oct. 2004	Average
Gazda				
1. Weedy control	7.8	35.1	57.4	33.4
2. Hoed control	5.1	14.8	37.2	19.0
3. Dual Gold + Callisto + Gesaprim (early post)		9.4	4.3	6.9
4. Merlin + Dezormon (early post)	1.5	10.9	4.1	5.5
5. Dual Gold + Callisto + Gesaprim (normal post)		2.6	4.8	3.7
6. Merlin + Dezormon (normal post)	1.6	2.4	6.0	3.3
7. Motivel + Cambio (normal post)	1.4	1.9	4.1	2.5
8. Titus 25 DF + Callisto + Trend (normal post)	1.1	5.9	6.3	4.4
9. Motivel + Cambio (late post)	2.3	15.7	14.0	10.7
10. Titus 25 DF + Callisto + Trend (late post)	2.2	16.3	16.9	11.8
Maraton				
1. Weedy control	8.4	29.7	56.2	31.4
2. Hoed control	4.9	15.3	39.6	19.9
3. Dual Gold + Callisto + Gesaprim (early post)		8.4	4.0	6.2
4. Merlin + Dezormon (early post)	1.7	7.8	3.9	4.5
5. Dual Gold + Callisto + Gesaprim (normal post)		2.9	4.9	3.9
6. Merlin + Dezormon (normal post)	1.7	2.0	6.3	3.3
7. Motivel + Cambio (normal post)	1.2	2.4	3.9	2.5
8. Titus 25 DF + Callisto + Trend (normal post)	1.0	6.3	6.8	4.7
9. Motivel + Cambio (late post)	2.3	14.2	14.5	10.3
10. Titus 25 DF + Callisto + Trend (late post)	2.1	13.9	16.8	10.9
Norma				
1. Weedy control	8.4	30.8	52.3	30.5
2. Hoed control	5.0	16.2	37.6	19.6
3. Dual Gold + Callisto + Gesaprim (early post)		10.2	4.2	7.2
4. Merlin + Dezormon (early post)	1.5	12.5	3.7	5.9
5. Dual Gold + Callisto + Gesaprim (normal post)		2.3	5.7	4.0
6. Merlin + Dezormon (normal post)	1.5	1.7	6.9	3.4
7. Motivel + Cambio (normal post)	1.1	1.9	3.5	2.2
8. Titus 25 DF + Callisto + Trend (normal post)	1.4	5.9	7.1	4.8
9. Motivel + Cambio (late post)	2.2	15.3	13.9	10.5
10. Titus 25 DF + Callisto + Trend (late post)	2.2	15.3	15.4	11.0

The rates of phytotoxicity resulting from herbicide treatments are included in Tables 5 and 6 for each year and hybrid. The data in the tables demonstrate that in the majority of cases no phytotoxicity was found in any treatments in any year. In a small number of hybrids and with certain herbicides a very moderate degree of phytotoxicity (2–6%) was observed. Side by side with visible phytotoxicity, however, herbicides may induce physiological processes resulting in considerable yield decreases, as confirmed by the results of the experiment (e.g. in the case of the hybrid Borbála there was a yield decrease in Treatments 9 and 10 compared with optimally timed herbicide applications). These latent physiological effects of herbicides depended greatly on the hybrid.

Table 5
Phytotoxic effect of herbicides on maize (%) (Debrecen, 2002–2004)

Hybrids and herbicide treatments	14 May 2002	13 May 2003	10 May 2004	Average
De 377 SC				
1. Weedy control	0	0	0	0
2. Hoed control	0	0	0	0
3. Dual Gold + Callisto + Gesaprim (early post)		0	3	2
4. Merlin + Dezormon (early post)	0	0	5	2
5. Dual Gold + Callisto + Gesaprim (normal post)		0	0	0
6. Merlin + Dezormon (normal post)	0	0	0	0
7. Motivel + Cambio (normal post)	0	0	0	0
8. Titus 25 DF + Callisto + Trend (normal post)	0	0	0	0
9. Motivel + Cambio (late post)	0	0	0	0
10. Titus 25 DF + Callisto + Trend (late post)	0	0	0	0
Veronika				
1. Weedy control	0	0	0	0
2. Hoed control	0	0	0	0
3. Dual Gold + Callisto + Gesaprim (early post)		0	0	0
4. Merlin + Dezormon (early post)	0	0	0	0
5. Dual Gold + Callisto + Gesaprim (normal post)		0	0	0
6. Merlin + Dezormon (normal post)	0	0	0	0
7. Motivel + Cambio (normal post)	0	0	0	0
8. Titus 25 DF + Callisto + Trend (normal post)	0	0	0	0
9. Motivel + Cambio (late post)	0	0	0	0
10. Titus 25 DF + Callisto + Trend (late post)	0	0	0	0
Borbála				
1. Weedy control	0	0	0	0
2. Hoed control	0	0	0	0
3. Dual Gold + Callisto + Gesaprim (early post)		0	5	3
4. Merlin + Dezormon (early post)	0	0	6	2
5. Dual Gold + Callisto + Gesaprim (normal post)		0	0	0
6. Merlin + Dezormon (normal post)	0	0	0	0
7. Motivel + Cambio (normal post)	0	0	0	0
8. Titus 25 DF + Callisto + Trend (normal post)	0	0	0	0
9. Motivel + Cambio (late post)	0	0	0	0
10. Titus 25 DF + Callisto + Trend (late post)	0	0	0	0

The annual and average yields achieved each year for the individual hybrids as the result of the herbicide treatments are presented in Tables 7 and 8. An analysis of the yields revealed that the amount of grain harvested was equally influenced by the year and the hybrid, as well as by the herbicide treatment applied. The best yields were obtained in the wetter cropping years of 2002 and 2004, while the yields in the drier year of 2003 were 1–2 t ha^{-1} lower.

Year	Weedy control yield (kg ha^{-1})	Yields in herbicide treatments (kg ha^{-1})
2002	9,875	9,043–10,374
2003	6,796	7,519–9,4660
2004	6,226	9,459–10,395

Table 6
Phytotoxic effects of herbicides on maize (%) (Debrecen, 2002–2004)

Hybrids and herbicide treatments	14 May 2002	13 May 2003	10 May 2004	Average
Gazda				
1. Weedy control	0	0	0	0
2. Hoed control	0	0	0	0
3. Dual Gold + Callisto + Gesaprim (early post)		0	2	1
4. Merlin + Dezormon (early post)	0	0	3	1
5. Dual Gold + Callisto + Gesaprim (normal post)		0	0	0
6. Merlin + Dezormon (normal post)	0	0	0	0
7. Motivel + Cambio (normal post)	0	0	0	0
8. Titus 25 DF + Callisto + Trend (normal post)	0	0	0	0
9. Motivel + Cambio (late post)	0	0	0	0
10. Titus 25 DF + Callisto + Trend (late post)	0	0	0	0
Maraton				
1. Weedy control	0	0	0	0
2. Hoed control	0	0	0	0
3. Dual Gold + Callisto + Gesaprim (early post)		0	0	0
4. Merlin + Dezormon (early post)	0	0	2	1
5. Dual Gold + Callisto + Gesaprim (normal post)		0	0	0
6. Merlin + Dezormon (normal post)	0	0	0	0
7. Motivel + Cambio (normal post)	0	0	0	0
8. Titus 25 DF + Callisto + Trend (normal post)	0	0	0	0
9. Motivel + Cambio (late post)	0	0	0	0
10. Titus 25 DF + Callisto + Trend (late post)	0	0	0	0
Norma				
1. Weedy control	0	0	0	0
2. Hoed control	0	0	0	0
3. Dual Gold + Callisto + Gesaprim (early post)		0	2	1
4. Merlin + Dezormon (early post)	0	0	3	1
5. Dual Gold + Callisto + Gesaprim (normal post)		0	0	0
6. Merlin + Dezormon (normal post)	0	0	0	0
7. Motivel + Cambio (normal post)	0	0	0	0
8. Titus 25 DF + Callisto + Trend (normal post)	0	0	0	0
9. Motivel + Cambio (late post)	0	0	0	0
10. Titus 25 DF + Callisto + Trend (late post)	0	0	0	0

The effects of the herbicide applications on the yields of the hybrids are shown in Figures 1, 2 and 3.

The extent of weed infestation influenced the yields of maize considerably. The rate of weediness in the weedy control group (Treatment 1) was very moderate in 2002 (7.6–8.4%), due to wheat as forecrop, leading to a very high yield (9875 kg ha^{-1}). The influence of the herbicide treatments was moderate in this year (0–500 kg ha^{-1} extra yield). In 2004, a wet year like 2002, the weed cover in the weedy control (Treatment 1) was 52.3–57.4% (with maize as forecrop), resulting in a considerable yield decrease (3200–4200 kg ha^{-1}) in comparison to the herbicide treatments. The rate of weed infestation in the weedy control in 2003 (Treatment 1) was more moderate as a result of the drier weather (27.4–35.1%), but owing to the less favourable water regime the yields were also moderate (6796 kg ha^{-1}). The extra yields achieved as a result of the herbicide treatments ranged between 700 and 2700 kg ha^{-1}.

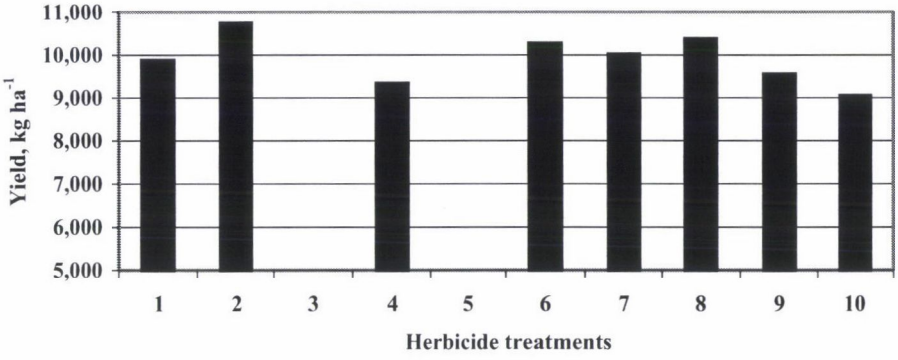

Fig. 1. Effect of herbicides on the yields of maize. Averaged over hybrids (Debrecen, 2002)

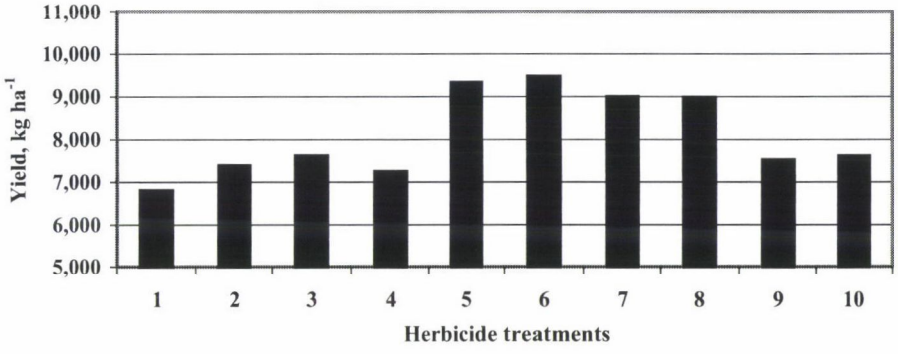

Fig. 2. Effect of herbicides on the yields of maize. Averaged over hybrids (Debrecen, 2003)

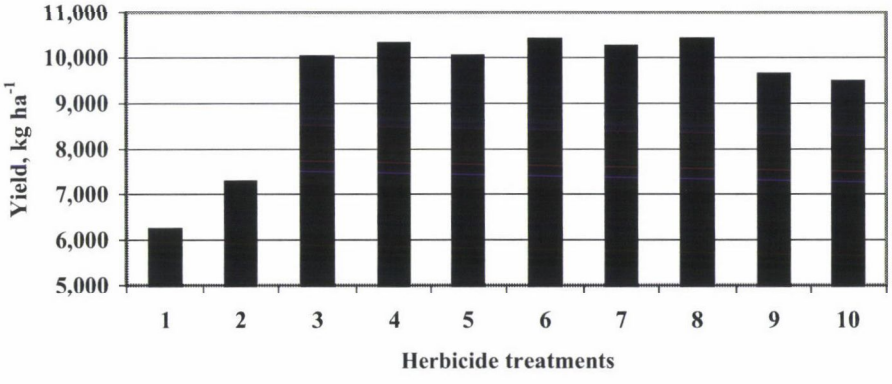

Fig. 3. Effect of herbicides on the yields of maize. Averaged over hybrids (Debrecen, 2004)

Table 7
Effects of herbicides on the yields of maize (kg ha^{-1}) (Debrecen, 2002–2004)

Hybrids and herbicide treatments	2002	2003	2004	Average
De 377 SC				
1. Weedy control	8,807	6,480	6,214	7,167
2. Hoed control	10,258	6,652	7,242	8,051
3. Dual Gold + Callisto + Gesaprim (early post)		7,944	9,555	8,750
4. Merlin + Dezormon (early post)	9,266	7,879	9,866	9,004
5. Dual Gold + Callisto + Gesaprim (normal post)		8,919	8,968	8,944
6. Merlin + Dezormon (normal post)	9,977	8,627	9,990	9,531
7. Motivel + Cambio (normal post)	9,976	8,693	10,930	9,866
8. Titus 25 DF + Callisto + Trend (normal post)	10,433	8,870	10,096	9,800
9. Motivel + Cambio (late post)	9,344	6,969	9,741	8,685
10. Titus 25 DF + Callisto + Trend (late post)	8,453	6,691	9,027	8,057
Veronika				
1. Weedy control	10,455	8,541	7,793	8,930
2. Hoed control	11,094	9,022	8,606	9,574
3. Dual Gold + Callisto + Gesaprim (early post)		8,847	11,072	9,960
4. Merlin + Dezormon (early post)	9,653	7,540	12,237	9,810
5. Dual Gold + Callisto + Gesaprim (normal post)		10,942	10,684	10,813
6. Merlin + Dezormon (normal post)	10,893	11,359	10,095	10,782
7. Motivel + Cambio (normal post)	10,128	10,001	10,083	10,071
8. Titus 25 DF + Callisto + Trend (normal post)	10,648	11,094	10,524	10,755
9. Motivel + Cambio (late post)	10,098	9,133	10,783	10,005
10. Titus 25 DF + Callisto + Trend (late post)	10,107	9,016	10,648	9,924
Borbála				
1. Weedy control	9,449	5,978	4,303	6,577
2. Hoed control	9,860	6,455	5,876	7,397
3. Dual Gold + Callisto + Gesaprim (early post)		5,797	9,363	7,580
4. Merlin + Dezormon (early post)	8,214	5,750	9,660	7,875
5. Dual Gold + Callisto + Gesaprim (normal post)		8,528	9,436	8,982
6. Merlin + Dezormon (normal post)	9,229	8,810	10,087	9,375
7. Motivel + Cambio (normal post)	9,206	8,917	9,483	9,202
8. Titus 25 DF + Callisto + Trend (normal post)	9,429	7,957	9,273	8,886
9. Motivel + Cambio (late post)	7,456	6,593	7,799	7,283
10. Titus 25 DF + Callisto + Trend (late post)	7,051	6,964	7,655	7,223

Despite the extremely moderate visible phytotoxicity, the sensitivity of the hybrids to the herbicide treatments manifested itself in the yields. The variety Borbála proved to be the most sensitive to late herbicide applications (general sensitivity to herbicides), but in certain years specific sensitive responses were also experienced with hybrids Gazda, Norma, Veronika and De 377 SC (specific sensitivity to herbicides).

On average, herbicide treatments led to 1–4 t ha^{-1} higher yields in comparison to mechanical weed control, depending on the hybrid and the year, proving the efficiency of herbicide applications. The sensitivity of the hybrids to herbicides and the optimum timing of herbicide applications, however, must also be taken into account.

Table 8
Effects of herbicides on the yields of maize (kg ha^{-1}) (Debrecen, 2002–2004)

Hybrids and herbicide treatments	2002	2003	2004	Average
Gazda				
1. Weedy control	9,447	6,471	6,549	7,489
2. Hoed control	10,514	7,378	7,971	8,621
3. Dual Gold + Callisto + Gesaprim (early post)		6,380	9,784	8,082
4. Merlin + Dezormon (early post)	10,263	5,947	9,892	8,701
5. Dual Gold + Callisto + Gesaprim (normal post)		8,516	10,557	9,537
6. Merlin + Dezormon (normal post)	9,812	8,984	10,426	9,741
7. Motivel + Cambio (normal post)	10,062	8,767	10,497	9,775
8. Titus 25 DF + Callisto + Trend (normal post)	9,513	8,366	10,867	9,582
9. Motivel + Cambio (late post)	9,281	7,605	9,942	8,943
10. Titus 25 DF + Callisto + Trend (late post)	8,802	7,594	9,934	8,777
Maraton				
1. Weedy control	12,531	7,612	6,486	8,876
2. Hoed control	13,022	8,022	7,229	9,424
3. Dual Gold + Callisto + Gesaprim (early post)		9,250	10,771	10,011
4. Merlin + Dezormon (early post)	10,152	9,284	10,686	10,041
5. Dual Gold + Callisto + Gesaprim (normal post)		10,347	10,648	10,498
6. Merlin + Dezormon (normal post)	12,166	10,167	11,430	11,254
7. Motivel + Cambio (normal post)	10,690	9,121	10,980	10,264
8. Titus 25 DF + Callisto + Trend (normal post)	12,698	9,255	11,550	11,168
9. Motivel + Cambio (late post)	10,874	8,815	10,434	10,041
10. Titus 25 DF + Callisto + Trend (late post)	10,835	8,988	10,245	10,023
Norma				
1. Weedy control	9,445	5,693	6,010	7,049
2. Hoed control	10,178	6,787	6,683	7,883
3. Dual Gold + Callisto + Gesaprim (early post)		7,466	9,515	8,491
4. Merlin + Dezormon (early post)	8,670	7,066	9,450	8,395
5. Dual Gold + Callisto + Gesaprim (normal post)		8,653	9,872	9,263
6. Merlin + Dezormon (normal post)	10,097	8,850	10,342	9,763
7. Motivel + Cambio (normal post)	10,136	8,442	10,288	9,622
8. Titus 25 DF + Callisto + Trend (normal post)	10,049	8,306	10,069	9,475
9. Motivel + Cambio (late post)	10,047	5,998	9,050	8,365
10. Titus 25 DF + Callisto + Trend (late post)	9,812	6,372	9,243	8,476

Discussion

Weed control is a very important element in the production technology of maize. In stands overgrown with weeds the effects and efficiency of various elements of crop management (soil cultivation, fertilization, irrigation, etc.) may be less than optimal. Owing partly to agricultural management, the weather and the weed composition, the importance of post-emergence weed control has gained in importance recently. The sensitivity of the numerous maize hybrids of different genotypes to herbicides shows a varied pattern.

Various maize hybrids were tested over three years with herbicide treatments containing different active agents applied in different ways. The

studies revealed that the extent of weed infestation was considerably modified by the forecrop. In the weedy control treatment (Treatment 1) the extent of weed infestation was 7.6–8.4% after wheat as forecrop and 27.4–35.1 and 52.2–57.4% after maize. The rate of weediness was much lower after a favourable forecrop (wheat) than after a less favourable one (maize). After the same forecrop (maize) the extent of weediness was largely defined by the effects of the year and the water supply. The weedy control treatment (Treatment 1) showed 27.4–35.1% weed infestation in the drier year of 2003, while in 2004, which had a more favourable water supply, this rate was much higher (52.3–57.4%). In the same treatments the hybrids only had a minimal effect on the extent of weed infestation.

In the majority of years the herbicide treatments resulted in little (2–6%) or no visible phytotoxicity in the hybrids. However, their latent effects on physiological processes became evident as a decrease in yield.

The effect of herbicide treatments on the yield depended on the year, the forecrop and their weed control efficiency. Depending on the herbicide and the hybrid, the yield-increasing effects of the herbicide treatments ranged from 700–2700 kg ha^{-1} in the moderately infested year (2003) and from 3200–4200 kg ha^{-1} in the heavily infested year (2004) as compared to 0–500 kg ha^{-1} (weakly infested year, 2002) in the weedy control (Treatment 1). The results demonstrate that the variety Borbála showed a marked sensitivity to post-emergence treatments (a general sensitivity to herbicides), while hybrids Gazda, Norma, Veronika and De 377 SC only showed sensitivity to certain herbicides or when herbicides were applied outside normal timing, and responded with yield decreases (specific sensitivity to herbicides).

Acknowledgements

This work was carried out with funding from the research fund of the Ministry of Education (OM 4/0008/2002).

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Acta Agronomica Hungarica, 53(1), pp. 31–39 (2005)

EFFECT OF WEATHER ON MAIZE YIELDS AND THE EFFICIENCY OF FERTILIZATION

L. HUZSVAI and J. NAGY

CENTER OF AGRICULTURAL SCIENCES, DEBRECEN UNIVERSITY, DEBRECEN, HUNGARY
HAS-DU LAND USE AND REGIONAL DEVELOPMENT RESEARCH TEAM, DEBRECEN, HUNGARY

Received: 17 March, 2005; accepted: 25 May, 2005

The yield of maize is primarily influenced by sunlight, temperature, available plant nutrients and water supply. Since plants take up water through their roots, the most decisive factor is not precipitation but the quantity of water available in the soil. In this study, a simple, easy-to-reproduce, capacitive model was elaborated to determine the available moisture content for maize. During the calculations, based on the balance method, the available moisture content in the top 110 cm soil layer was determined, taking daily weather data into account. The examinations were carried out on a medium heavy chernozem soil with lime deposits, in a multifactorial experiment at the Látókép Experimental Station of the Center of Agricultural Sciences, Debrecen University, between 1990 and 2004. Annual yield fluctuation is primarily determined by the soil moisture content in the month of July and the water supplies in May, according to regression analysis. The maize yields in the past 15 years could be calculated with an accuracy of 570 kg/ha, an error limit of below 10% and an r value of 0.805, using a regression line and the data of monthly moisture supplies. However, the yields of fertilized plots can only be estimated with an accuracy of 1 t/ha on average. Fertilizer utilization is influenced by the moisture content of the soil, so it makes sense to include this in the analysis instead of the other environmental factors. Water is required for nutrient utilization. In years with poor or medium water supplies, moderate fertilizer rates are more effective, compared to higher rates in years with better water supplies. Efficient fertilization in maize production can only be achieved by harmonizing soil moisture content and the applied fertilizer rate.

Key words: maize, fertilizer, yield estimation, models

Introduction

The high degree of climate change is one of the most important risk factors in crop production, and must be constantly taken into account even within the framework of modern agriculture. For decades, or even centuries, climate has been assumed to be stable, though this contradicts all the laws of nature, since, like the planet Earth itself, the atmosphere is constantly being transformed, though the rate of change is very slow and difficult to measure. This explains the fact that climate was previously regarded as a constant factor. This view has now changed and climate change is mentioned more and more frequently. In different years, deviation in the temperature and in the quantity and distribution of precipitation may significantly influence yields even under very similar growing conditions.

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The number of droughty and extremely wet years has increased in the past hundred years. Both have a harmful effect on field crop production and on the extent to which it can be planned. Bocz (2001) also highlighted the deterioration in precipitation supplies. Barrov et al. (2000) investigated changes in precipitation quantities in the winter period (+0.4 – +3.6%) and in the summer period (–0.5 – +3.7%) in Europe between 1961 and 1990. Láng (1976) and Márton (2002) drew attention to the role of weather in influencing yields. Berényi (1956) also regarded the quantity of precipitation as a decisive factor, having found that natural water supplies were responsible for 55–75% of fluctuations in yields. A close relationship was detected between the "year effect", plant nutrient supplies and the yield (Csathó et al., 1991; Kádár, 1992; Jolánkai et al., 1999; Márton, 2000).

Since the weather regulates the heat and moisture supplies of the growing site, it has an effect on material transformations in the soil, on the growth and nutrient uptake of plants and thus on fertilizer utilization (Kramer, 1963; Kovács, 1982). Nagy and Huzsvai (1995) examined the effect of year on the efficiency of fertilization using a fourteen-year series of yield data for maize hybrids and found that fertilization improved rainfall utilization to a greater degree in the growing season than in the winter season. Fertilization also improves rainfall utilization in absolute terms. According to Berzsenyi and Győrffy (1997), in dry years the effect of lower fertilizer rates was more stable. Yield decreases were recorded in dry years at high nitrogen levels due to the high salt concentration (Debreczeni and Debreczeni, 1983). In moderately dry years a medium or good fertilizer effect was observed in most experiments, and under such conditions P and K supplies were of great significance due to the decrease in water deficiency stress. Rainfall quantities and the moisture stored in the soil modify both fertilizer requirements and fertilizer effects. The fertilizer effect increases as optimal water supplies are approached and decreases again when water supplies become excessive (Szász, 1972; Bocz, 1976; Ruzsányi, 1992). Both deficiencies and excessive nutrient supplies increase drought sensitivity (Ruzsányi, 1996). Optimal N supplies contribute significantly to an increase in grain number per ear and to a smaller extent to an increase in thousand kernel weight (Bocz and Nagy, 1981). Dry matter accumulation, however, is smaller and the dynamics of dry matter accumulation is slower in maize plants when N deficiency occurs (Hanway and Russell, 1969; Berzsenyi, 1993; Dobos et al., 1999; Dobos and Nagy, 1999). A rapid increase in maize leaf area in the early phase of development is promoted by adequate N supplies, so optimal LAI values and biomass can be sustained for longer periods, which is favourable for assimilate flow to the grain yield, leading to a favourable harvest index (Anderson et al., 1985; Berzsenyi, 1988). In dry years, however, this advantage does not lead to any economic benefit, because the maize suffers from water deficiency at an earlier stage, which reaches its peak during the reproductive period, thus resulting in yield losses.

Materials and methods

The multifactorial experiments were set up on medium heavy chernozem soil with lime deposits at the Látókép Experimental Station of the Faculty of Agriculture, Debrecen University.

The experimental station is located west of the Pece stream in Látókép, between the stream and a track at an elevation of 113–118 masl. The highest point of the area can be found in the NW corner of the station, with a 1% slope to the SE. The surface of the area undulates, with elevated points rising to around 116.6–117.2 m, and hollows of around 115.8 m. The majority of the soil is lowland calcareous chernozem, with leached chernozem on some lower-lying areas acting to some extent as catchment areas. The soil texture is heavy clay. The total porosity of the cultivated layer and the 10–15 cm layer beneath it is 44–48%, while that of the layer below 40 cm is 50–53%, which is typical of chernozem soils. The ratio of gravitational pores significantly decreases in the 10–15 cm layer beneath the cultivated layer. As a consequence, the permeation of water from the cultivated layer is slow. The humus layer is 70–80 cm thick, of which the top 30 cm has a humus content of 2.5–3.0%. The $pH_{(KCl)}$ of the cultivated layer averages 6.2. On the basis of total nitrogen content the N supplies of the area are moderate, while the phosphorus and potassium supplies are good. This soil description was based on soil analysis carried out at the start of the experiment.

Fertilizer treatments

A constant NPK ratio of 1 N : 0.75 P_2O_5 : 0.88 K_2O was applied with a basic rate of 80 kg/ha (including 30 kg/ha N). The treatments involved 1, 2, 3, 4 and 5 times this rate, along with an unfertilized control. The experiment was arranged in strips, with the hybrids and fertilizer treatments perpendicular to each other in four replications. In the present study, the data recorded for hybrids in the FAO 300 maturity group grown in unirrigated plots between 1990 and 2004 were analysed.

Evaluation

The evaluations were carried out using the SPSS for Windows 11.0 statistical software package. Linear regression analysis was used to estimate the yields in unfertilized treatments, and non-linear regression analysis to forecast the yields of fertilized treatments. The sum of squares was minimized during approximation in both methods. The Levenberg-Marquardt method was chosen for fitting the non-linear function. The r-value and the size of the estimation error were used to determine the goodness of fit. The function parameters were tested with the t-probe, at the 5% significance level.

Results and discussion

In Hungary the yield of maize is primarily influenced by sunlight, temperature, available plant nutrients and water supply. The water supply depends on soil characteristics, as well as on the quantity and spatial distribution of precipitation. Since plants take up water through their roots, the quantity of water available in the soil is a more decisive factor than the quantity of rainfall, though naturally it is greatly influenced by precipitation levels. Thus, in order to forecast yields, it is essential to continuously monitor the moisture content of the soil. This can be achieved through measurements or modelling. Continuous measurements, carried out at various locations, are very expensive and time-consuming, so computer programs that model the water balance in the soil are becoming widespread. However, these models are often too complicated for

farm experts, requiring great technical knowledge and previous measurements. The present work was aimed at developing a simple, easy-to-reproduce, capacitive model to determine the moisture content available for maize.

During the calculations, based on the balance method, the available moisture content in the 110 cm soil layer was determined from daily weather data. The calculations were based on the following parameters: the average field water capacity of the soil profile, the non-available water content, and the potential and actual evaporation. The method of Szász (1971) was used to determine the potential evaporation, and an algorithm elaborated by the authors for the actual evaporation. The daily change in soil moisture content provides a good description of years. Two different years are illustrated in Figure 1.

To estimate the yields of fertilized plots the yield of unfertilized maize was first modelled as a function of the water content. To simplify the calculations, the mean available moisture content of the 100 cm soil profile was determined for the months of May, June, July and August. Since yield is formed by the dry matter accumulation over a period of several months, the following simple additive linear function was chosen:

$$Y = b_0 + b_1 DV_V + b_2 DV_{VI} + b_3 DV_{VII} + b_4 DV_{VIII}$$

where:

Y	: yield (t/ha)
$b_0 ... b_4$	: coefficients of the regression line
$DV_V ... DV_{VIII}$	: available water content from May to August (mm)

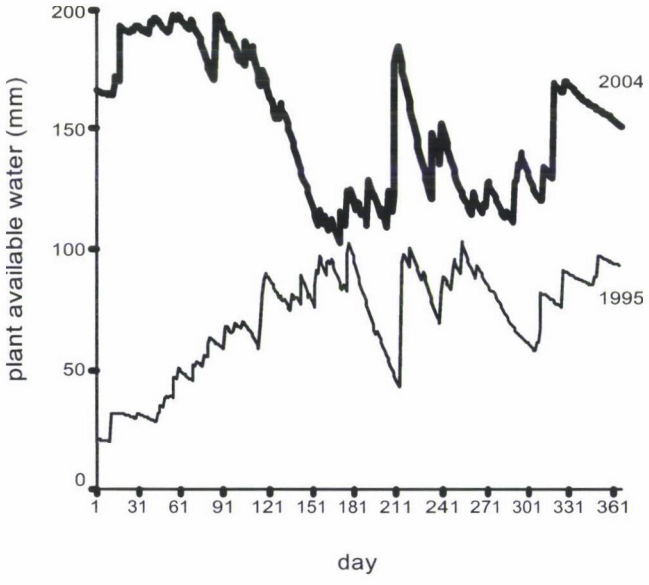

Fig. 1. Available water reserves in the 110 cm soil layer, Debrecen, 1995, 2004

The results of data fitting are presented in Table 1. Standardized values indicate that the annual yield fluctuation is primarily determined by soil moisture content in July, followed by the water supplies in May. The soil moisture content in June and August did not change significantly during the period examined (1990–2004), so it was not worth including it in the analysis. The maize yields obtained over the past fifteen years can be calculated with an accuracy of 570 kg/ha with the help of the regression line, representing an error of less than 10% (0.805). Figure 2 depicts the estimated and measured values. In order to check the results, the calculations were also performed using the monthly precipitation quantities. The estimation error then increased nearly threefold, to 1650 kg/ha. This confirms that the yield of maize is primarily influenced by the water-supplying capacity of the soil, rather than by the amount of precipitation.

Table 1
Results of regression analysis of maize yield

	Unstandardized coefficients		Standardized coefficients
	B	Std. error	Beta
b_0	3.142	0.496	
b_1	8.975E–03	0.005	0.263
b_2	–2.163E–03	0.010	–0.071
b_3	1.658E–02	0.009	0.651
b_4	6.387E–06	0.005	0.000

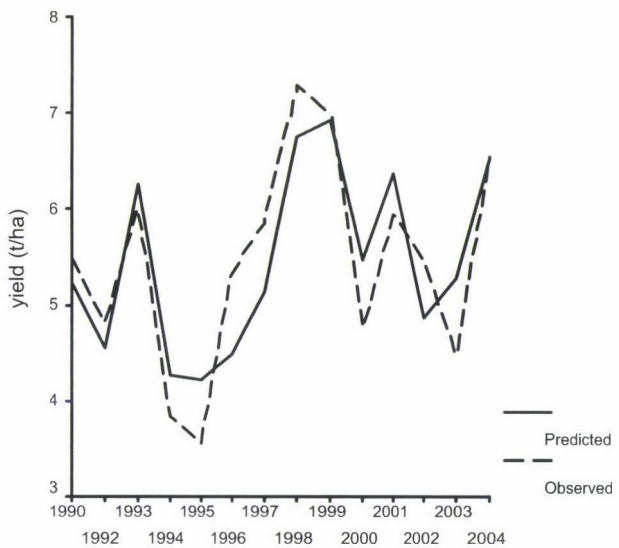

Fig. 2. Estimated and measured yields in unfertilized maize, Debrecen, 1990–2004

Based on the results of long-term experiments carried out for several decades, the yield-increasing effect of fertilization is known to vary from year to year, depending primarily on the water-supplying capacity of the soil. Figure 3 demonstrates the maize yields achieved over the past nine years as a function of fertilization. It can be clearly seen that when yields are low, because the water supply is unfavourable for maize, higher fertilizer rates often cause yield depression. In years with good water supplies, higher fertilizer rates further increase the yield, and depression is rarely or never observed. In this case, instead of the quadratic function often applied, the correlation can be more accurately described with a saturation function. The question is, whether a universal function accurately describing the effect of fertilization on the yield for the examined period, can be constructed. For this purpose the quadratic function generally used was transformed to take into account the very varied annual and monthly water reserves of the soil.

The modified function is as follows:

$$Y = b_1 DV_V + b_2 DV_{VII} + b_3 N + b_4 N^2$$

where:

Y : yield of fertilized maize (t/ha)

$b_1 ... b_4$: the coefficients of the regression line

$DV_V ... DV_{VII}$: the available water content in May and July (mm)

Table 2 presents the results of the regression analysis. The yields of fertilized plots can be estimated with a mean accuracy of 1 t/ha. This error margin is far greater than in the case of unfertilized plots (multiple r value: 0.72). A more accurate result (with an error margin of 100–200 kg/ha) can only be obtained if the parameters of the quadratic function are determined annually, but in this case the result cannot be generalised and the function cannot be used for forecasting yields. It can thus be concluded that, even with the present, modern cultivation technologies, the weather significantly modifies the efficiency of fertilization in increasing yields. For the time being, the accuracy of prediction cannot be reduced below 1 t/ha, if a simple, functional model is used to describe long-term correlations. However, the relationship between weather and fertilization can be well illustrated even with such a simple model. Among the weather components, precipitation is utilized through the soil. It is the momentary moisture content of the soil that influences the utilization of fertilizer rather than the quantity of precipitation, so this factor should be included in the analysis. Nutrient utilization requires water, so more nutrients require more water. In years with poor or moderate water supplies, moderate fertilizer rates are efficient, while in years with good water supplies higher rates can be utilised (Fig. 3). The efficient fertilization of maize can only be achieved by harmonizing the soil moisture content and the fertilizer quantities applied.

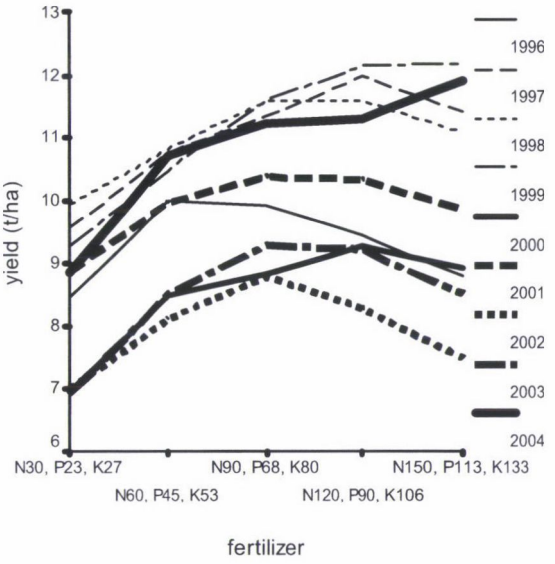

Fig. 3. Maize yields as a function of fertilization, Debrecen, 1996–2004

Table 2
Results of regression analysis of maize yield

	Unstandardized coefficients	
	B	Std. error
b_1	0.0283	0.00782
b_2	0.0137	0.00679
b_3	0.1092	0.01394
b_4	–0.0005	0.00008

Acknowledgements

This research was carried out with the support of the project OM 4/0008/2002 and the Hungarian Academy of Sciences – Land Use Research Team, Debrecen University.

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Acta Agronomica Hungarica, 53(1), pp. 41–46 (2005)

IRRIGATION OF MAIZE (*ZEA MAYS* L.) IN RELATION TO FERTILIZATION IN A LONG-TERM FIELD EXPERIMENT

A. MEGYES, J. NAGY, T. RÁTONYI and L. HUZSVAI

DEPARTMENT OF LAND USE AND RURAL DEVELOPMENT,
CENTRE OF AGRICULTURAL SCIENCES, DEBRECEN UNIVERSITY, DEBRECEN, HUNGARY

Received: 21 March, 2005; accepted: 10 May, 2005

The objectives of this study were to examine the correlation between factors of great significance for crop production, especially between irrigation and fertilization, and to evaluate the effects of irrigation and fertilization on maize yields over four growing seasons (2001–2004) in a long-term field experiment set up at the Látókép Experimental Station of the Centre for Agricultural Sciences of Debrecen University. The results showed that irrigation and fertilization were strongly correlated with the yield. The effect of irrigation depended on the natural water and nutrient supplies of the soil, and on the specific fertilizer rates. The results indicated that both fertilization and irrigation had a significant effect on the yield. The correlation between the year and the crop production factors was also significant. The yield-increasing effect of irrigation and fertilization differed significantly in the experimental years.

Key words: maize yield, irrigation, fertilization

Introduction

Papers in both Hungarian and foreign journals agree that, among the factors influencing the fertilizer effect, the weather, soil characteristics, water supplies, a uniform stand, and the fertilizer response of the crop are the most decisive. Since the weather regulates the heat and moisture supply of the growing site, it has an effect on the transformation of materials in the soil and on the growth and nutrient uptake of plants, thus influencing fertilizer efficiency (Kramer, 1963; Kovács, 1982; Nagy, 1996). The amount of precipitation and the moisture stored in the soil modifies both the need for fertilization and the fertilizer effect. The fertilizer effect increases when the water supplies approach the optimal level and decreases in the case of excess water (Nagy, 1994). Irrigation and fertilization experiments carried out by Nagy (1995; 1997) proved that irrigation increases the efficiency of fertilization and there is a strong correlation between fertilizer utilization and the water supply of plants. In irrigated treatments, which lead to higher yield levels, economical fertilizer rates are greater than without irrigation, due to the positive correlation between irrigation and fertilization. In irrigated cultivation the effect of the year is moderate and yield fluctuations decrease.

Research has proved that irrigation generally increases the yield of maize, especially in the case of drought. If precipitation and the readily available water supplies in the soil do not satisfy the needs of the plant, the deficiency must be

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compensated for with irrigation (Petrasovits, 1978). An accurate irrigation forecast can only be given in the knowledge of the precipitation and soil moisture conditions of the plot (Balogh, 1978). Irrigation is becoming more and more important in some parts of the country for the safe, intensive production of maize (Szőke Molnár, 1977). The period from the second half of the 1980s until the mid-1990s, which was extremely droughty, drew particular attention to the need for irrigation.

The objectives of this study were to examine the correlations between factors that are of significance for crop production, especially between irrigation and fertilization, and to evaluate the effects of irrigation and fertilization on maize yields over four growing seasons (2001–2004).

Materials and methods

Experimental site

This study was conducted over four years, from 2001 to 2004, at the Látókép Experimental Station of the Centre for Agricultural Sciences, Debrecen University located in the north-eastern part of the Great Hungarian Plain (47°30'N, 21°36'E, 121 m elevation). The investigations were part of a multi-factorial long-term field experiment set up without replication in a split-split-plot design with tillage and irrigation treatments in the main plots, maize hybrids sown at plant densities of 50, 70 and 90 thousand plants ha^{-1} in the split plots, and fertilization treatments in four random replications in the split-split plots. The size of each tillage block was 8064 m^2, split into an irrigated and a non-irrigated block. Tillage treatments were winter ploughing to a depth of 27 cm, spring ploughing to a depth of 22 cm and shallow spring disk tillage to a depth of 12 cm. Fertilizers were applied at a rate of 0, 120, 240 kg ha^{-1} N, 0, 90, 180 kg ha^{-1} P, and 0, 106, 212 kg ha^{-1} K.

The examinations were carried out on unfertilized and fertilized (N_{120}, N_{240} kg ha^{-1}) plots, in the non-irrigated and irrigated treatments. During irrigation, an amount of water close to the needs of the maize stand was applied.

Soil characteristics

The soil of the experimental site was a lowland pseudomyceliar chernozem (Mollisol-Calciustoll or Vermustoll, silt loam; USDA taxonomy). The soil had moderate N and P supplies and a high K content (humus content 2.8–3.0%, total N 0.14–0.18%, Al-P_2O_5 130–200 mg ha^{-1}, Al-K_2O 240–280 mg ha^{-1}). The thickness of the humus layer was 70–90 cm. The pH_{KCl} value was 6.2; the upper limit of plasticity according to Arany was 43. No microelement deficiency was detected. The groundwater level was between 4–5 m. The field capacity of the soil was 27–29 vol%. The 0–100 cm soil layer was able to retain 275 mm of moisture, while the 100–200 cm could store 265 mm. The available water content was 157 mm in the 0–100 cm soil layer and 150 mm in the 100–200 cm layer.

Weather characteristics

The weather in 2001 and 2004 was very favourable for maize, with sufficient precipitation both in the winter and in the vegetation period. The rainfall distribution was also favourable, especially in the period of July and August, which is so critical for the development of maize plants. In 2002, there was 104 mm less precipitation in the winter season and 92 mm less during the growing season than the fifty-year average. The weather conditions in 2003 were similar to the previous year, with 158 mm less precipitation than the mean annual value (Fig. 1).

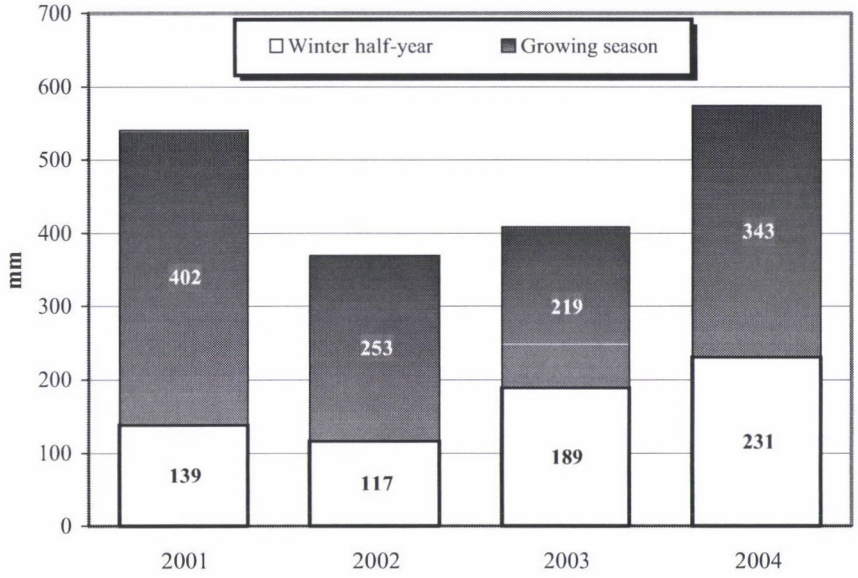

Fig. 1. Distribution of precipitation in winter and during the growing season (Debrecen, 2001–2004)

Statistical evaluation

The research program was designed to allow reliable evaluation, using an improved version of the Box and Wilson (1951) method. The experimental data were evaluated with variance analysis (Sváb, 1981). The 11.0 version of the SPSS for Windows was used to evaluate the data.

Results

The results showed that irrigation and fertilization were strongly correlated with the yield. The effect of irrigation depended on the natural water and nutrient supplies of the soil, and on the specific fertilizer rates. The results of analysis showed that both fertilization and irrigation had a significant effect on the yield. The correlation between the year and the crop production factors tested was also significant. The yield-increasing effect of irrigation and fertilization differed significantly in the years examined. The results are presented in Table 1.

An evaluation of the effect of fertilization indicated that in the dry years of 2002 and 2003, without irrigation, the highest fertilizer rates caused yield depression. According to the results of variance analysis, fertilization significantly decreased the yield of maize. In the N_{240} kg ha^{-1} treatment the maize yield was 9–10% lower than at a fertilizer rate of N_{120} kg ha^{-1}.

Table 1
Results of analysis of variance (Debrecen, 2001–2004)

Source of variance	SS	df	MS	F	Sig
Corrected model	14459.9	17	850.6	127.8	0.000
Intercept	206354.7	1	206354.7	30997.0	0.000
Year	9817.0	3	3272.3	491.5	0.000
Irrigation	551.5	1	551.5	82.8	0.000
Fertilization	3549.7	2	1774.9	266.6	0.000
Year × irrigation	173.4	3	57.8	8.7	0.000
Year × fertilization	560.4	6	93.4	14.0	0.000
Irrigation × fertilization	192.0	2	96.0	14.4	0.000
Error	22727.8	3414	6.7	–	–
Total	249411.5	3432	–	–	–
Corrected total	37187.7	3431	–	–	–

In 2002 and 2003, the yield of maize increased significantly under irrigated conditions, with a surplus yield of around 0.6–2.9 t ha^{-1}, depending on the nutrient supply level. The greatest irrigation effect was achieved on the plot fertilized with 240 kg ha^{-1} N in both years. On the other hand, the efficiency of irrigation was very low under unfertilized conditions and a slight yield decrease was observed in 2002 (Fig. 2).

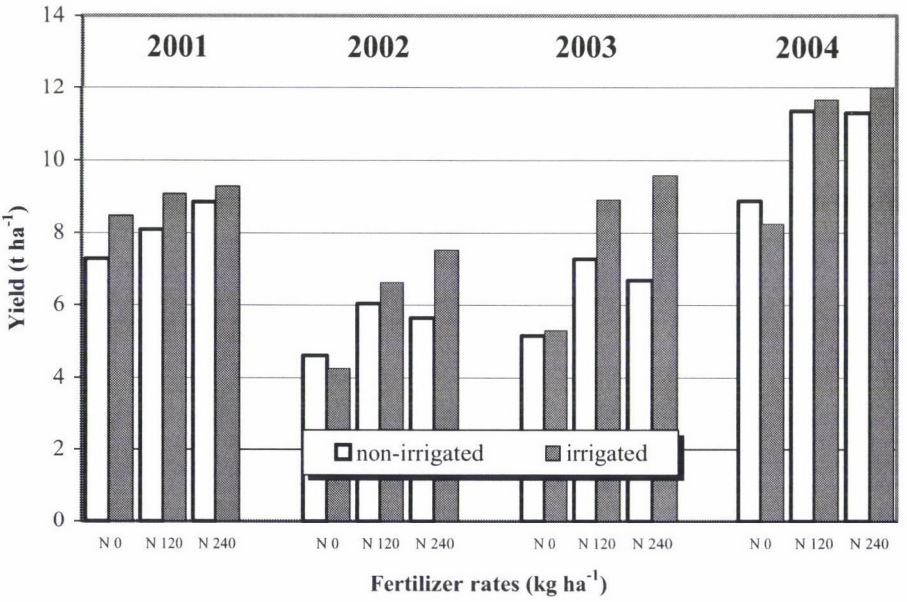

Fig. 2. Effect of fertilization on maize yields under irrigated and unirrigated conditions (Debrecen, 2001–2004)

The years 2001 and 2004 were favourable as regards precipitation for maize, and fertilization significantly increased the yield even without irrigation, averaged over four years. The surplus yield achieved with fertilization was 0.8–2.4 t ha^{-1} depending on the treatment level. The surplus yield reached a maximum on plots where the lower 120 kg N rate was applied. There was no further significant increase at the 240 kg N ha^{-1} rate.

Due to the effect of irrigation, the yield was significantly higher at all three nutrient supply levels compared to the unirrigated treatments. The highest fertilizer dose, as under unirrigated conditions, did not increase the yield of maize significantly. The significant year or year × irrigation correlation confirmed that the yield surplus achieved by irrigation in 2001 and 2004 significantly differed from the irrigation effect measured in 2002 and 2003.

Discussion

When evaluating the results it was found that fertilization and the year significantly influenced the efficiency of irrigated maize production. The experimental data confirmed the results of previous research indicating that the amount of precipitation and the moisture stored in the soil modify the fertilizer effect, so that less fertilizer is needed at lower water supply levels.

According to the results of the experiment, the application of 240 kg N ha^{-1} is not recommended under any conditions, since it did not significantly increase the yield of maize compared to the 120 kg ha^{-1} N rate even with irrigation. In droughty years it caused a significant yield decrease. This N fertilizer rate clearly resulted in over-fertilization and decreased the profitability of maize production. In addition, it represents a constant, potential source of danger, since in irrigated cultivation high rates of N fertilizer may multiply the amount of nitrate leached from the root zone, increasing the pollution of the groundwater. When determining the optimal amount of irrigation, the amount of N fertilizer applied must be considered as well as the NO_3 content of the soil, in order to avoid leaching from the upper layer.

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Acta Agronomica Hungarica, 53(1), pp. 47–52 (2005)

ANALYSIS OF THE RIPENING DYNAMICS OF MAIZE HYBRIDS ON CHERNOZEM SOIL WITH LIME DEPOSITS

A. C. DOBOS

HAS–DU LAND USE AND REGIONAL DEVELOPMENT RESEARCH TEAM,
CENTER OF AGRICULTURAL SCIENCES, DEBRECEN UNIVERSITY, DEBRECEN, HUNGARY

Received: 18 March, 2005; accepted: 25 May, 2005

Maize is one of the most important crops in Hungarian crop production, making up 70–72% of forage consumption. Due to high energy prices and the rapidly increasing costs of drying, the need for maize hybrids with shorter growing seasons is increasing. Farmers require hybrids with a grain moisture content of 20–25% at harvest, making them suitable for storage with less drying.

In order to avoid excessive costs and workloads during harvest, it is wise to cultivate a mixture of hybrids with longer and shorter growing seasons as well as with slower and faster drying down rates.

The effect of fertilization on the yield components and moisture content of maize was examined, using the data from a two-year survey on the hybrid MV 484 SC, with five fertilizer treatments in 1998, and a control and three (0, 90 and 150 kg N/ha) nutrient levels in 1999.

Key words: maize, fertilization, moisture content, yield components

Introduction

The aim of field crop production is to produce plant products in sufficient amounts and appropriate quality. The quantity and composition of the organic matter produced is determined by the amount of assimilates produced during photosynthesis in the growing season.

After examining many hybrids Bellini and Fusi (1961) found that 43–49% of all dry matter in maize was located in the grain.

During the grain filling period the temperature and water supply have a significant impact (Kiesselbach, 1950). There is a positive correlation between temperature and the rate of grain growth (Duncan et al., 1965).

Grain reaches its dry matter maximum at the beginning of black layer formation (Carter and Poneleit, 1973), which is the indicator of physiological ripening (Daynard and Duncan, 1969). The black layer forms beneath the seed coat at the core, when the cells of the connective tissue die. These dead cells block matter flow, but certain substances can pass through the black layer, as found by Duffus and Cochran (1982).

Grain filling is accompanied by a change in the grain moisture content. In the active period, up to physiological ripening, physiological factors dominate. Path analysis (Kang et al., 1986) demonstrated a positive direct effect between the rate of grain filling and the water content % (on each growth day); faster

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grain filling was accompanied by faster water loss. Water loss is influenced by the grain type (Derieux, 1975; Derieux and Bonhomme, 1982), the thickness of the pericarp (Purdy and Crane, 1967), and the number and quality of the husks. Optimal levels of the majority of production technology components (plant density, fertilization), not only increase yields, but also decrease the grain moisture content (Kising, 1962; Nagy and Zeke, 1981). Adequate nutrient supplies to maize not only increase yields, but also improve water utilization (Kovács, 1982; Nagy, 1993; 1996; Németh and Búzás, 1991). When examining the effect of N fertilization on the moisture content of maize grains, Gagro (1974) found that grain moisture was the highest in the unfertilized and in the 200 kg/ha N active agent treatments, while Gotlin and Pucoric (1977; cit. Nagy and Zeke, 1981) found that at increasing fertilizer rates the grain moisture content decreased. These authors demonstrated on five hybrids that fertilization resulted in an increase in yield and crude protein content and a decrease in grain moisture content. Irrigation, especially in the case of low nutrient supplies, significantly increased the grain moisture of many hybrids (Nagy and Zeke, 1982).

Materials and methods

A long-term, three-factorial (fertilization, irrigation, genotype) experiment was set up at the Látókép Experimental Station of Debrecen University in 1978, as part of cooperation between the Center of Agricultural Sciences, Debrecen University and KITE PLC, Nádudvar. The research has been funded by the National Scientific Research Fund since 1991. The yield potential, natural nutrient utilization ability and fertilizer responses of 10 maize hybrids are examined each year in a split plot design with four replications, with the fertilizer treatments in the main plot (120 m^2) and the maize hybrids in the sub-plots (15 m^2). Plots of similar size were used for destructive sampling.

The Experimental Station is situated on the Hajdúság loess-back, and the soil is a lowland chernozem with lime deposits and a deep humus layer. The soil texture is medium hard loam. The groundwater is located at a depth of 5–8 m (Rátonyi, 1999).

The daily rainfall sum was determined by on-site measurements, while the daily radiation and temperature data were provided by the Agrometeorological Observatory of Debrecen University and the National Meteorological Service, Budapest. Among the agrometeorological parameters an analysis was made of precipitation during the growing season, the effective heat sums during the vegetative and generative phases, potential evaporation and the water supply. The daily heat sums were determined using the algorithm of Ritchie et al. (1994).

During the 1998 winter half-year there was 157 mm of precipitation, which was 86 mm less than the average of many years. However, 470.9 mm fell during the growing season (first ten days of May – first ten days of October), exceeding the 50-year average by 130.9 mm. The rainfall, along with the water resources stored in the soil, resulted in optimal water supplies.

Like 1998, 1999 was wetter than average, with a total precipitation of 635.4 mm, 389.2 mm of it during the growing season.

The effect of fertilization on the grain filling, grain number and drying down of MV 484 SC was investigated in an unfertilized control and five fertilizer rates in 1998, and in treatments with 0, 90 and 150 kg/ha N in 1999, with a constant NPK ratio of 1N : 0.75 P_2O_5 : 0.88 K_2O and a basic rate of 89 kg/ha.

Destructive samples were taken weekly in both years following flowering, and on each occasion the weight of 50 grains from the mid-section of 4 maize cobs was measured in four

replications. Dry matter content was determined after drying to constant weight in a drying cabinet at 60°C.

The effect of fertilization on grain filling, drying down and grain number was determined using regression analysis and the Duncan test according to Sváb (1981).

The statistical analysis was carried out using SPSS for Windows 9.0 and Excel XP softwares.

Results

The grain number increased significantly in the fertilized treatments, compared to the control plots, proving that the nutrient supply (at a given water supply) favourably influences fertilization and yield.

The multiple R values were characterised by a narrow interval (0.84–0.93), while the F probe proved the effect of fertilization to be significant at the 0.1% level in both years. The linear and squared terms in the equation were also significant at the 0.1% level on the basis of the t-probe. The linear element tended to be dominant in both years, while the squared term only had a minor depressive effect (Table 1).

The Duncan test was used to examine the significance of differences between fertilizer treatment means (Table 2). The grain number of MV 484 SC increased steadily, reaching a maximum in the 120 kg N/ha fertilizer treatment.

Fertilization promoted grain filling. No significant difference in the slope of the linear sections, i.e. in the rate of grain filling, was observed in the different treatments; fertilization increased the length of the grain filling period.

When examining the effect of fertilization on thousand grain weight at harvest it was found that better nutrient supplies significantly increased the thousand grain weight of MV 484 SC (Table 3).

Grain filling took 320 degree days in 1998, averaged over the treatments, while in 1999, due to the favourable water supplies, the linear phase of the grain filling period was 100 degree days longer.

Table 1

Results of regression analysis on MV 484 SC grain number (Debrecen, 1998–1999)

	Variance analysis				
	SS	df	MQ	F	Sig F
Regression	219087.45	2	109543.72	58.32198	0.0000
Remainder	39443.42	21	1878.26		
	Regression analysis				
Factors	Coefficients	SE B	Beta	t	Sig T
Constant	223.596211	19.639594		11.385	0.0000
Fertilizer	5.011911	0.615786	2.474101	8.139	0.0000
Fertilizer square	–0.022941	0.003941	–1.769736	–5.822	0.0000

Multiple R: 0.92056; Multiple R^2: 0.84743; Standard error: 43.33887

Table 2
Duncan test on the grain number of MV 484 SC (Debrecen, 1998 – 1999)

Fertilizer treatment	N	α = 0.05			
		1	2	3	4
2	7	277			
30	4		349		
60	4			426	
150	7			479	479
90	7			487	487
120	4				527
Sig.		1.000	1.000	0.091	0.182

Table 3
Variance analysis on the thousand-grain weight of MV 484 SC (Debrecen, 1998–1999)

1998	SS	df	MQ	F	Sig F
Fertilizer	20181.062	5	4036.212	5.749	0.002
Error	13339.888	19	702.099		
Total	33520.950	24			
1999	SS	df	MQ	F	Sig F
Fertilizer	20963.742	2	10481.871	32.213	0.001
Error	1952.373	6	325.396		
Total	22916.116	8			

An analysis of the effect of fertilization on the grain moisture content in different stages of grain development (Fig. 1) showed that there was no significant difference between the treatments in either year at the beginning of the generative phase. From the end of August 1998, however, significant differences were found in the grain moisture contents of different treatments after a value of 50% water content, proving the effect of fertilization.

The grain moisture content decreased at physiological ripening as a result of fertilization. The lowest moisture content was recorded in the 60 kg N/ha fertilizer treatment in 1998, while higher fertilizer treatments increased the moisture content. In 1999 this value decreased significantly up to a fertilizer rate of 150 kg N/ha.

Lower moisture contents were recorded at physiological ripening in 1999 than in 1998, due to the favourable year. This can be explained by the late formation of the black layer, while grain filling was not hampered by any external factor.

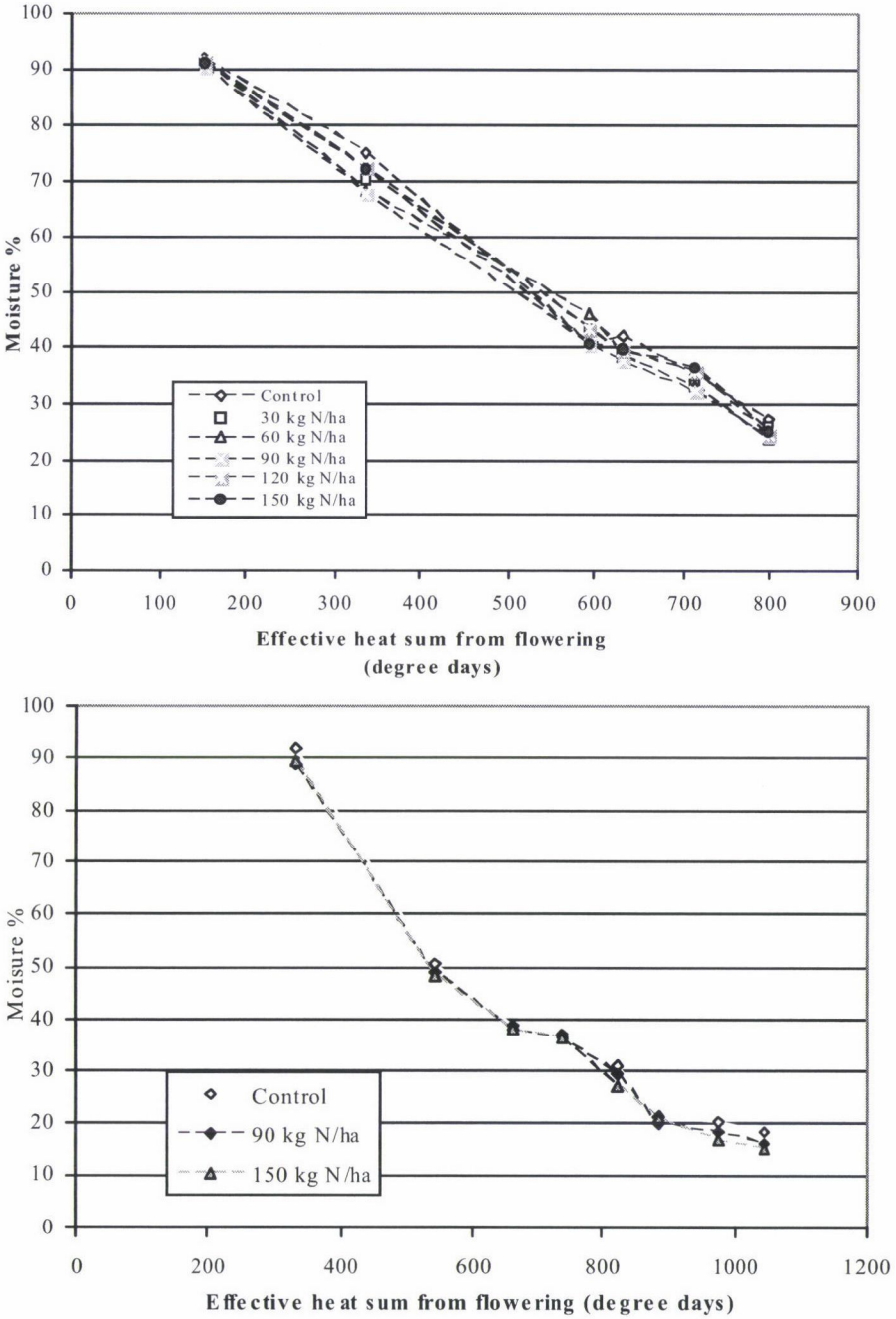

Fig. 1. Effect of fertilization on the drying down dynamics of MV 484 SC (Debrecen, 1998–1999)

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Acta Agronomica Hungarica, 53(1), pp. 53–57 (2005)

EVALUATION OF SOIL TILLAGE SYSTEMS IN MAIZE PRODUCTION

T. RÁTONYI, L. HUZSVAI, J. NAGY and A. MEGYES

DEPARTMENT OF LAND USE AND RURAL DEVELOPMENT,
CENTRE OF AGRICULTURAL SCIENCES, UNIVERSITY OF DEBRECEN, DEBRECEN, HUNGARY

Received: 17 March, 2005; accepted: 18 April, 2005

The cultivation technologies for the dominant crops in Hungary need to be improved both in the interests of environmental protection and to reduce cultivation costs. A long-term research project was initiated in order to determine the feasibility of conservation tillage systems. The aim of the experiments was to evaluate conservation farming systems in Hungary in order to achieve more economical and more environment-friendly agricultural land use.

Four tillage systems, namely conventional tillage (mouldboard plough), conservation tillage I (primary tillage with a J.D. Disk Ripper), conservation tillage II (primary tillage with a J.D. Mulch Finisher) and no tillage (direct drilling), were compared on a clay loam meadow soil (Vertisol). The physical condition of the experimental soils was evaluated using a hand-operated static cone penetrometer. Parallel with the measurement of penetration resistance, the moisture content of the soil was also determined. The grain yield of maize hybrids (Kincs SC [1999], Occitán SC [2000], Pr 37M34 SC [2001], DeKalb 471 SC [2002]) was measured using a plot combine-harvester. The analysis of soil conditions confirmed that if the cultivation depth and intensity are reduced the compaction of soil layers close to the surface can be expected. The decrease in yields (8–33%) in direct drilling (NT) and shallow, spring cultivated (MF) treatments, despite the higher available water content, can be explained partly by the compacted status of the 15–25 cm soil layer.

Key words: conventional tillage, conservation tillage, direct drilling, water content, penetration resistance, maize yield

Introduction

The cultivation technologies for the crops dominant in Hungary need to be improved both in the interests of environmental protection and to reduce cultivation costs. It is a well-known fact that tillage is one of the most energy-demanding processes in crop production. The processes of soil loosening and ploughing make up about 30–40% of the energy demand of tillage and in extreme cases this figure may reach 50%. Farmers and scientists all over the world have been trying to reduce the intensity of the mechanical manipulation of the soil and the number of tillage operations.

Conventional tillage methods in Hungary are usually followed by soil deterioration, soil compaction and a decrease in the organic content, and these side-effects have caused increasing economic and environmental problems (Birkás et al., 1989; Gyuricza, 2000). Experiments have proved that maize yields do not decrease significantly, inputs can be reduced, and the same or even 20% higher profit can be achieved in maize production (Nagy, 1995).

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The aim of the experiments was to evaluate conservation farming systems in Hungary in order to achieve more economical and more environment-friendly agricultural land use.

Materials and methods

The research was carried out on a large-scale farm in Csárdaszállás (46° 50' N, 20° 55' E, 90 m above sea level) on a clay loam meadow soil (Vertisol). The research area was divided into four plots each of 2.8 ha (320 × 90 m). Four tillage systems, namely conventional tillage (CT, mouldboard plough), disk ripper (DR), mulch finisher (MF) and no tillage (NT) were compared. Details of the tillage treatments and the type of primary tillage operations applied to the experimental plots are listed below:

- Conventional tillage (winter ploughing to a depth of 27 cm, CT)
- Conservation tillage I (primary tillage with John Deere 510 disk ripper to a depth of 30 cm, DR)
- Conservation tillage II (primary tillage with a John Deere mulch finisher to a depth of 15 cm, MF)
- Direct drilling (NT) with a John Deere 1750 seed drill

The physical status of the experimental soils was evaluated using a hand-operated static cone penetrometer. Parallel with the measurement of penetration resistance the moisture content of the soil was also determined. The grain yield of maize hybrids (Kincs SC [1999], Occitán SC [2000], Pr 37M34 SC [2001], DeKalb 471 SC [2002]) was measured using a plot combine-harvester. In the present study, the results obtained from the sample site in the years 1999–2002 were evaluated.

Results

Compaction and moisture status of the soil

On the basis of soil resistance values recorded using a penetrometer various compacted layers were detected in the soil profile. From the compactness of the soil, conclusions were drawn on the loosening effect of the cultivation tools, on differences in looseness between the various cultivated layers, and on the physical status of the soil below the ploughed layer.

When the intensity and depth of cultivation were reduced there was a substantial increase in the compaction of soil layers close to the surface. The disk ripper proved to be a satisfactory tool for the elimination of the increasingly thick compacted layer developing below the ploughed layer as the result of tillage in previous years (Fig. 1). The greatest degree of compaction close to the soil surface was recorded in the case of direct drilling and shallow cultivation in spring (mulch finisher), due principally to the lack of deep ploughing and to the concentration of effects leading to compaction close to the soil surface. As the result of traditional soil preparation involving soil turning, the looseness of the soil in the cultivated layer was satisfactory, but below it an extremely compacted plough-sole layer developed.

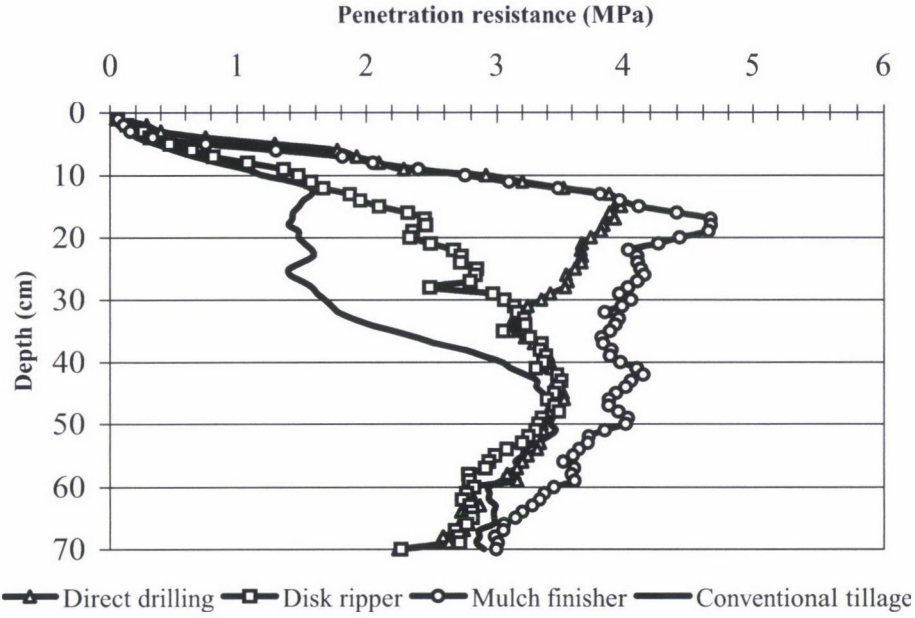

Fig. 1. Effect of tillage systems on the penetration resistance of the soil (Csárdaszállás, 2004)

Moisture profiles drawn on the basis of moisture content measurements during the vegetation period indicated that the different treatments led to significant differences in the soil moisture content in the upper 1.8 m soil profile, which increased in the following order: CT < DR < MF < NT. Direct drilling proved to be the most favourable for the preservation of soil moisture content (Fig. 2). The moisture status of the soil clearly confirmed the favourable moisture preservation achieved by cultivation without soil turning compared to cultivation based on traditional ploughing.

Maize yields achieved with traditional and conservation soil cultivation technologies

On the basis of field measurements the tillage methods had a great influence on the maize yield. The conventional tillage (CT) system resulted in a significantly higher mean maize yield due to the higher plant population and grain weight per cob. Among the conservation tillage treatments the disk ripper (DR) provided the best conditions for maize growth and development. The maize yield was 3–12% lower on the DR plot in comparison with the CT system. The yields in the NT and MF treatments were 8–33% lower than in CT, which can be attributed to the significantly lower plant density and harder soil.

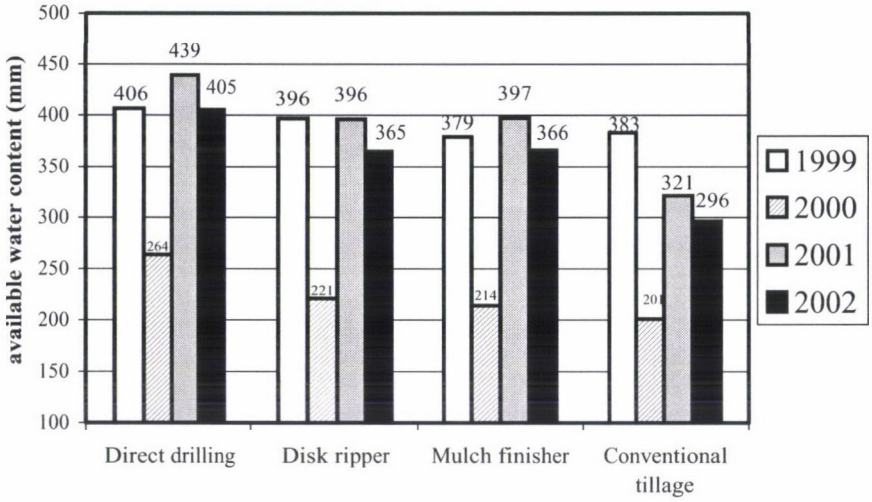

Fig. 2. Effect of tillage systems on the available water content in the 0–180 cm soil profile (Csárdaszállás, 1999–2002)

An assessment of the yields in the four experimental years shows that the yields achieved using a disk ripper (DR) are close to the results of conventional tillage (CT). The four-year average maize yield in the no tillage (NT) treatment was only 1.47 t ha^{-1} lower than after conventional tillage (CT), proving the importance of reduced tillage technologies (Fig. 3).

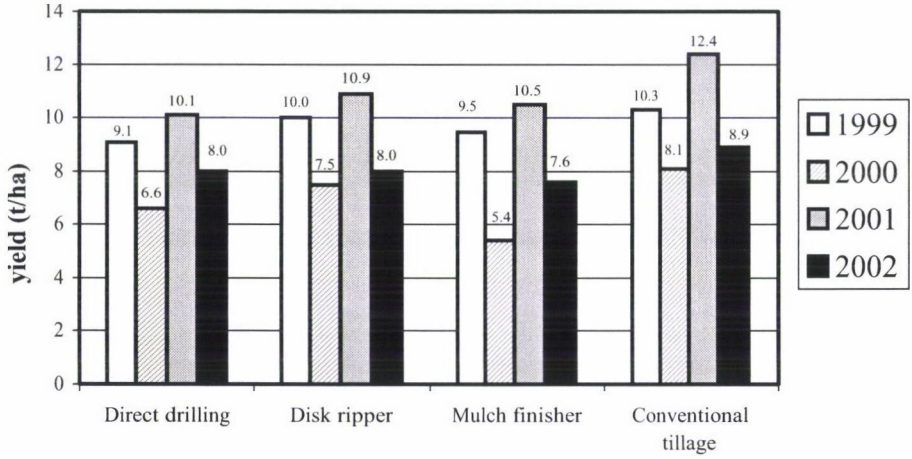

Fig. 3. Effect of tillage systems on the yield of maize (Csárdaszállás, 1999–2002)

Discussion

It was confirmed on the basis of soil status analysis that with a reduction in the cultivation depth and intensity (mouldboard plough ⇒direct sowing), the compaction of soil layers close to the surface can be expected to increase, while the omission of soil turning will improve the moisture content available. The decrease in yields on directly sown (NT) and shallow, spring cultivated plots (MF), despite the higher available water content, can be explained partly by the compacted status of the 15–25 cm soil layer. The experience gained with the disk ripper, among the basic tillage tools used in soil cultivation technologies, has been very favourable. The tool proved to be easy on soil structure, while significantly improving the physical condition of the soil, and it could be an important component in moisture-saving technologies, allowing yield levels similar to those achieved with traditional technologies. Soil and plant analyses carried out during the evaluation of traditional and conservation tillage systems have confirmed the farm applicability of maize production technologies based on unploughed, conservation tillage systems.

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Acta Agronomica Hungarica, 53(1), pp. 59–70 (2005)

IMPACT OF NUTRIENT SUPPLY, SOWING TIME AND PLANT DENSITY ON MAIZE YIELDS

M. SÁRVÁRI

DEPARTMENT OF CROP PRODUCTION AND APPLIED ECOLOGY,
CENTRE OF AGRICULTURAL SCIENCES, UNIVERSITY OF DEBRECEN, DEBRECEN, HUNGARY

Received: 18 March, 2005; accepted: 11 May, 2005

In order to enhance the adaptability and yield stability of maize, the effect of nutrient supply and plant density on yield was studied on a calcareous chernozem soil in Debrecen, while the relationship between sowing date and the grain moisture content at harvest was investigated on a typical meadow soil in Hajdúböszörmény.

In the plant density experiment, the plant densities applied were 45, 60, 75 and 90 thousand plants/ha.

The optimal fertilizer rates for the maize hybrids were: N 40–120, P_2O_5 25–75, K_2O 30–90 kg ha^{-1}.

The application of NPK fertilizers in a wet year increased the yield by 40–50%. Hybrids with good chilling resistance at germination can be sown as early as 10 April, when the soil temperature reaches 8–10°C.

There was a significant correlation between sowing date and the grain moisture content at harvest. When hybrids with good chilling resistance at germination were sown early, the grain moisture content at harvest was reduced by 5–10%.

A plant density higher than the optimum reduces yield and yield stability. The optimal plant densities determined in the experiment were 60, 75 and 90 thousand plants/ha for two, three and one hybrid, respectively.

Key words: sowing date, plant density, hybrid-specific, grain moisture content, yield

Introduction

Maize yields are largely dependent upon nutrient supply, sowing date and plant density. The effect of these three factors is also influenced by the close correlation between ecological and biological factors.

In earlier years Hungary was one of the leading countries in the world as regards the yields achieved in maize production (Menyhért, 1979), but at present besides unfavourable changes in climatic conditions, the level of NPK fertilization has also decreased.

In the 1980s, 278 kg/ha NPK active ingredients were applied, which decreased to 37 kg/ha by 1991. In 2002, the amount applied was 70 kg/ha, 80–90% of which was N, while P and K replenishment was neglected. Kádár (2000) drew attention to the fact that fertilization should aim at supplementing deficient nutrients. Therefore, when planning fertilization, the amount of nutrients removed by the yield should be taken as the basis. According to Sárvári (1995), if both efficacy and environmental aspects are considered, the most favourable rate of N for maize on meadow soil is 60–120 kg/ha active ingredients, depending on the forecrop and the year.

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In experiments at Martonvásár from 1988 until 1992, Berzsenyi (1993) obtained maximum maize biomass production and the highest absolute growth rate in treatments given 160 or 240 kg N/ha. The biomass production and growth rates recorded in these treatments did not significantly differ in rainy years.

Based on the results of several years, Sárvári (1986) stated that, besides nitrogen, potassium is the most important nutrient on meadow soil.

Debreczeni (1990) claimed that a proper potassium supply increased photosynthetic activity, making it important for both yield quantity and quality.

Menyhért (1978) found that the amount of nutrient uptake by maize during the vegetation period was N 264 kg/ha, P_2O_5 110 kg/ha and K_2O 264 kg/ha at a grain yield of 11 t/ha.

Németh and Széll (1985) mentioned that the variety policy followed in Hungary is open to varieties from all over the world. The cultivation of better varieties or hybrids accounts for 50% of the yield increase.

Optimal sowing time is largely influenced by climatic and soil conditions and is determined by the chilling resistance of the hybrid at germination.

According to the results of Balás and Hensch (1889), Fleischmann (1938), I'só (1959) and Pásztor (1958), the following factors should be taken into consideration when determining the optimal sowing time of maize: changes in temperature during the vegetation period, the date and frequency of frosts in late spring and early autumn, the composition and location of the soil, weed infestation, infection by pests and pathogens, the quality of the seed and its temperature requirements during germination and development, seed resistance to harmful factors, the possibility of seed dressing against pathogens and pests, the vegetation period of the hybrid to be grown, the response of the hybrid to external factors, the aim of production, field size, the machinery available for sowing and the sowing method.

I'só (1966) indicated that it is much more difficult to determine the optimal sowing time for maize than for other spring cereals. He attributed this primarily to the higher temperature requirements of maize during germination. The results of experiments in Martonvásár showed that the yield of maize sown in mid-April was 7% higher than that of maize sown in mid-May. The results indicated that a one-month delay in sowing resulted in the plants maturing 11–16 days later (14 days on average).

In experiments on the growth dynamics of 5 hybrids with different vegetation periods, Berzsenyi et al. (1998) found that earlier sowing enhanced reproductive growth, while later sowing increased the early vegetative growth. They also determined that a one-week delay in sowing resulted in a 3-week delay in silking.

Sárvári and Futó (2001) found a close correlation between sowing time and both yield and grain moisture content at harvest. The relationship between sowing time and yield was highly influenced by the distribution of precipitation during the vegetation period. Earlier sowing reduced the grain moisture content at harvest by 5–8%, which is a great advantage economically.

Széll and Csala (1984) found that, besides the response of the hybrid to increased plant density, the achievement of optimal plant density was primarily dependent upon the water and nutrient supplies.

Széll et al. (1986) stated that an over-dense plant population resulted in water deficiency. In most cases it caused a reduction in yield and reduced yield stability.

The results of Ruzsányi (1987) indicated that maize stands of 80,000–90,000 plants/ha require 50–70 mm more water than less dense stands. Considering the water supplies currently available, moderation is advisable when determining the number of plants per hectare.

Berzsenyi (1994) stated that grain yield gradually increased until the optimal plant density was reached, after which it started to decrease. In wet and dry years, the optimal plant density of the same hybrid was 80,000 and 50,000 plants/ha, respectively. An increase in plant density resulted in a consistent reduction in the harvest index.

According to Sárvári (2001), due to the increase in the frequency of dry years and the lower nutrient supply, plant densities of 68,000–72,000 plants/ha and 60,000–65,000 plants/ha are more suitable for FAO 200–300 and FAO 400–500 hybrids, respectively, than the 80,000–90,000 plants/ha suggested in the seventies and eighties.

Bocz (1974) determined that among the three major nutrients, N rates are of primary importance in increasing yields. However, the efficiency of N fertilizer is determined not only by the soil characteristics, plant species and hybrid but also by ecological factors. A higher fertilizer rate may result in higher yields, but it can also have a greater negative effect under unfavourable conditions.

Materials and methods

The experiment set up to study the effects of plant density and fertilization was carried out on calcareous chernozem soil. The organic matter content of the soil was 2.57%. The upper layer of the soil was dry due to its deficiency in lime, and was prone to cracking in dry years. The ground water level was 7–9 m.

In the sowing time experiment the organic matter content of the meadow soil was 4.2% and the groundwater level was 2–2.5 m. The cultivated layer of the soil was prone to silting up in wet years and to cracking in dry years. It was difficult to cultivate, much of the P was bound in the soil, and the fertilizer response was poorer than average for N and better for PK.

The yield potential, fertilizer response and plant density response of six maize hybrids were studied on calcareous chernozem soil, while the relationship between sowing time and yield, and sowing time and grain moisture content at harvest was investigated on meadow soil from 2000 until 2004.

Table 1 shows the dates of sowing and harvest in the experiments. Table 2 contains the fertilizer rates (active ingredient kg/ha) applied in the fertilization experiments.

Table 1
Dates of sowing and harvest in the experiments

Experiment	2002		2003		2004	
	Date of sowing	Date of harvest	Date of sowing	Date of harvest	Date of sowing	Date of harvest
Fertilization experiment	Apr. 10–11	Sep. 26–27	Apr. 7	Sep. 22	Apr. 15–16	Oct. 17–18
Sowing time experiment I	Apr. 17	Oct. 10	Apr. 12	Oct. 3	Apr. 9	Oct. 2
II	Apr. 27	Oct. 10	Apr. 17	Oct. 3	Apr. 26	Oct. 2
III	May 7	Oct. 10	Apr. 25	Oct. 3	May 13	Oct. 2
IV	May 17	Oct. 10	May 17	Oct. 3	–	Oct. 2
Plant density experiment	Apr. 12	Sep. 26–27	Apr. 8–9	Sep. 21	Apr. 13	Sep. 21–22

Table 2
The fertilizer rates (active ingredient kg/ha) applied in the fertilization experiments

Treatment	N	P	K
1	0	0	0
2	40	25	30
3	80	50	60
4	120	75	90
5	160	100	120
6	200	125	150

In the plant density and sowing time experiments, the standard nutrient supply was 110 kg/ha N, 80 kg/ha P_2O_5 and 130 kg/ha K_2O.

The plant densities were 45, 60, 75 and 90 thousand plants/ha.

The dynamics of drying down in the grains was measured once a week during the ripening period.

In 2002 (Fig. 1), the amount of precipitation in the Debrecen region in the first nine months of the year and in the vegetation period was 456.5 mm and 373 mm, respectively, which was more than the average over 30 years (354.1 mm). In Hajdúböszörmény, the amount of precipitation in the first nine months of the year and in the vegetation period was higher (454.5 mm and 394.1 mm, respectively) and exceeded the thirty-year average.

In 2003, the weather in Debrecen was extreme, especially during the vegetation period of maize (from April to September). The annual precipitation was 414 mm. The amount of precipitation in the maize vegetation period was 44.5 mm lower than the thirty-year average and its distribution was unfavourable. In Hajdúböszörmény, both the amount and the distribution of the precipitation were unfavourable. The water deficiency was further intensified by the high daily mean temperatures. During the vegetation period of maize, the amount of precipitation was 78.5 mm lower than the average over thirty years. The lack of precipitation in April, May and June was eased by the rainy July.

The weather in Debrecen in 2004 was favourable on the whole, especially compared to the previous years of drought, though the rainfall distribution was not. The amount of precipitation in June, July and August was satisfactory and contributed to the favourable yields. The distribution of precipitation in Hajdúböszörmény was unfavourable in May, with 40 mm of rain up till 8 May and hardly any during the rest of the month (1.7 mm). June was very rainy (147.3 mm), which had a very advantageous effect on the yield.

The results were evaluated by analysis of variance and parabolic regression analysis.

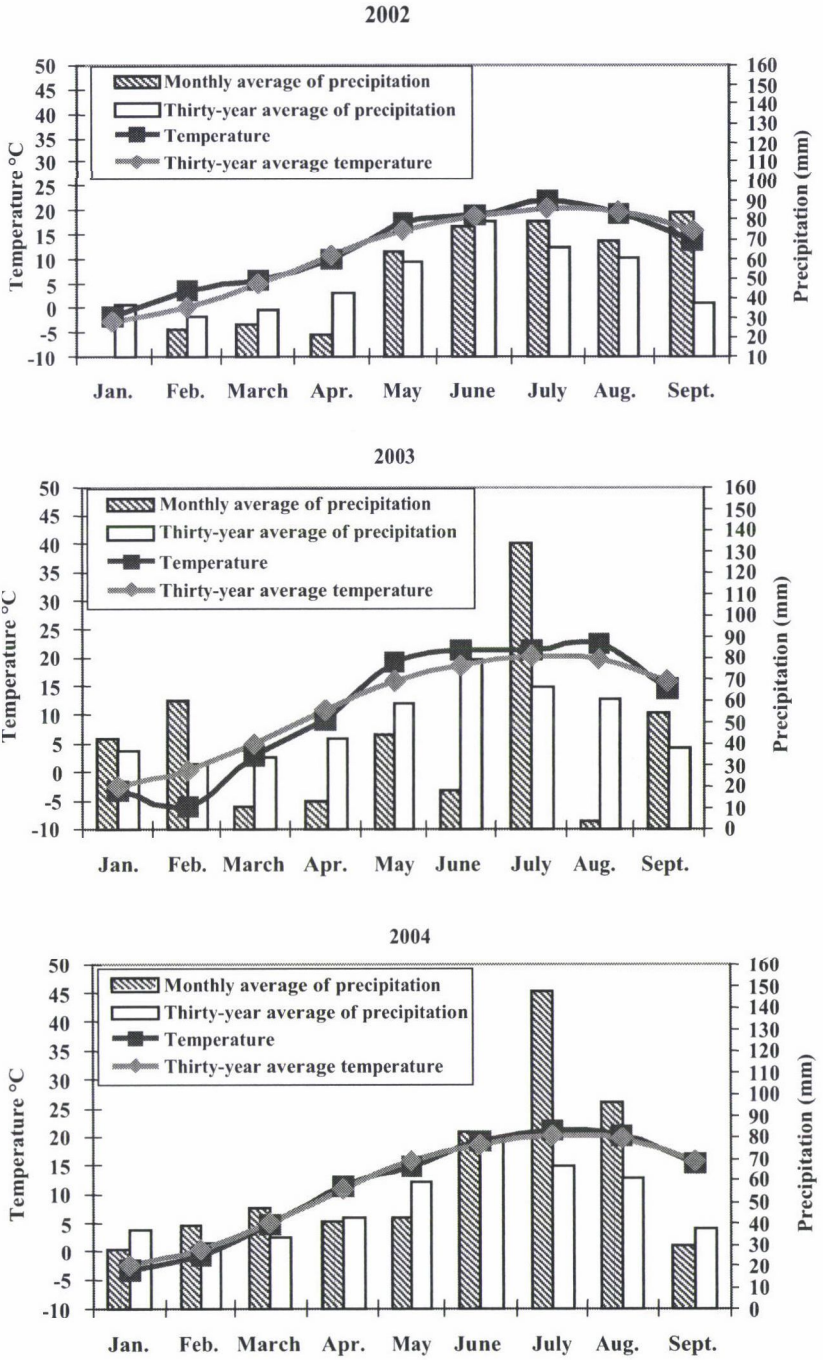

Fig. 1. Changes in the precipitation and temperature, Hajdúböszörmény

Results

The adaptability and yield stability of maize are determined by several factors. As a result of global warming, weather extremes are becoming more intense and the frequency of drought is increasing. Because of the unfavourable climatic factors, balanced NPK fertilization is of special importance.

There are great differences among the maize hybrids as regards yield potential, natural nutrient exploiting ability and fertilizer response.

The weather varied over the three experimental years, the amount of precipitation during the vegetation period of maize in 2002 being higher than the thirty-year average, though its distribution was unfavourable. There was a drought in 2003, while 2004 was wetter than average.

The yield in the unfertilized control (Table 3) was 2.31–3.12, 1.60–2.32 and 2.82–4.38 t/ha in 2002, 2003 and 2004, respectively. In 2002, a fertilizer rate of N 40, P_2O_5 25, K_2O 30 kg/ha increased the yield by 3.88–4.21 t/ha compared to the control. When twice this rate was applied, the yield increment was only 0.38–0.83 t/ha, while the triple rate (N 120, P_2O_5 75, K_2O 90 kg/ha) caused a slight (but not significant) decrease in yield.

In the dry year of 2003, the N 40, P_2O_5 25, K_2O 30 kg/ha rate increased the yield by 4.84–5.47 t/ha, while higher rates caused no further increase in the yield of the hybrids. The maximum yield was only 6.54–7.56 t/ha, compared with 9.73–12.41 t/ha in the more favourable, rainy year of 2004. In 2004, the N 40, P_2O_5 25, K_2O 30 kg/ha rate resulted in a yield increment of 4.15–7.70 t/ha and due to the favourable precipitation, even the N 120, P_2O_5 75, K_2O 90 kg/ha rate caused a significant 1.13–2.63 t/ha increase in yield compared to the N 80, P_2O_5 50 and K_2O 60 kg/ha rate.

Table 4 shows the maximum yield of the hybrids (t/ha) in the dry and favourable years.

It can be seen that hybrids with a longer vegetation period have higher yield potential, but this higher yield can only be achieved in favourable years.

The hybrids Mv SC 277 and Mv Vilma had the best natural nutrient exploiting ability, while Mv Maraton gave the best fertilizer response (Fig. 2).

In a dry year, a balanced ratio of NPK fertilizers has a more decisive role in yield stability than the quantity of fertilizer applied.

The optimal fertilizer rates for the maize hybrids were:

– in a dry year: N 40, P_2O_5 25 and K_2O 30 kg/ha

– in an average year: N 80, P_2O_5 50 and K_2O 60 kg/ha

– in a wet year: N 120, P_2O_5 75 and K_2O 90 kg/ha

The fertilizer efficiency decreased at increasing rates.

There is a strong correlation between sowing time and yield, but the close significant correlation between sowing time and grain moisture content at harvest is more important. Hybrids with a longer vegetation period have higher yield potential, but the advantage of this yield surplus may be diminished by the extra drying costs.

Table 3
Effect of fertilization on maize yields (t ha^{-1})

Hybrid	Fertilizer treatment*	Yield t ha^{-1}			Average
		2002	2003	2004	
Mv SC 277	Control	2.76	1.83	4.38	2.99
	1	6.64	7.3	7.34	7.09
	2	7.02	7.31	8.72	7.68
	3	6.82	6.35	11.35	8.17
	4	6.54	6.31	10.03	7.63
	5	7.29	6.04	9.46	7.60
Borbála	Control	2.29	1.6	3.03	2.31
	1	5.58	6.54	5.82	5.98
	2	6.41	6.04	6.6	6.35
	3	5.73	5.87	7.83	6.48
	4	5.47	5.47	8.24	6.39
	5	6.51	5.03	9.73	7.09
Debreceni 377	Control	1.88	1.93	3.18	2.33
	1	6.09	7.36	7.08	6.84
	2	6.16	7.24	8.61	7.34
	3	6.15	7.56	9.06	7.59
	4	6.17	7.14	9.27	7.53
	5	6.8	6.55	10.81	8.05
Mv Maraton	Control	2.48	2.01	3.14	2.54
	1	6.77	6.66	7.29	6.91
	2	7.48	6.86	8.65	7.66
	3	6.71	6.28	9.78	7.59
	4	7.13	6.04	12.13	8.43
	5	7.73	5.74	11.3	8.26
Szegedi 428	Control	2.73	2.32	2.82	2.62
	1	5.71	7.41	10.23	7.78
	2	5.48	7.05	10.54	7.69
	3	6.15	6.52	11.24	7.97
	4	6.03	6.54	11.72	8.10
	5	6.61	5.82	9.76	7.40
Mv Vilma	Control	3.29	2.23	3.83	3.12
	1	6.53	7.07	11.53	8.38
	2	6.45	6.49	11.44	8.13
	3	5.45	6.6	12.41	8.15
	4	5.73	5.66	10.39	7.26
	5	6.08	5.49	9.87	7.15
$LSD_{5\%}$		1.3	0.17	0.33	

* For fertilizer rates see Materials and Methods

Hybrids with a good cold test value (above 90%), i.e. with good chilling tolerance at germination, can be sown 10–15 days earlier than the conventional sowing time of 15 April–6 May. As a consequence of global warming, the soil temperature has been above 10°C by 5–10 April in recent years.

Early and very early hybrids have a wider optimal sowing date period than hybrids with a longer vegetation period.

Table 4
Maximum yield of hybrids (t ha^{-1}) in dry and favourable years

Hybrid	Yield in the dry year	Yield in the favourable year
Mv SC 277 (FAO 290)	6.35	11.35
Borbála (FAO 310)	6.04	9.73
Debreceni 377 (FAO 340)	7.56	10.81
Mv Maraton (FAO 450)	6.86	12.13
Szegedi 428 (FAO 460)	7.41	11.72
Mv Vilma (FAO 510)	6.60	12.41

In 2002–2003, the highest yield was obtained with a later (7–17 May) sowing date, because precipitation was lower than the average in May–June and higher in July. Late-sown stands were able to utilize the precipitation in July and August, but their moisture content at harvest was 6–12% higher (Table 5).

Mv Vilma responded sensitively (with a yield decrease) to earlier sowing dates.

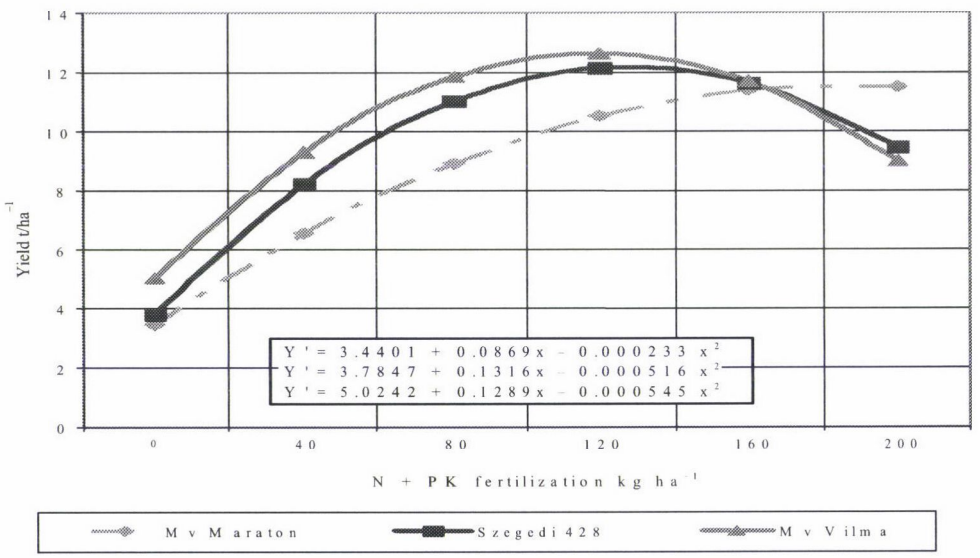

Fig 2. Relationship between fertilization and the yield of maize hybrids

Table 5
Effect of sowing date on the grain moisture content (%) at harvest in maize

Hybrid	Sowing date	Moisture content			Average
		2002	2003	2004	
Mv SC 277	Apr. 1–10	16.00	10.00	18.53	14.84
	Apr. 21–30	20.00	11.33	24.47	18.60
	May 1–10	22.00	11.33	25.00	19.44
	May 21–30	20.00	16.00	–	20.00
Borbála	Apr. 1–10	16.00	10.67	21.80	16.16
	Apr. 21–30	17.33	12.00	21.20	16.84
	May 1–10	26.67	11.33	25.27	21.09
	May 21–30	26.67	16.00	–	21.34
Debreceni 377	Apr. 1–10	18.00	12.00	25.27	18.42
	Apr. 21–30	22.67	12.67	26.00	20.45
	May 1–10	32.00	14.00	32.07	26.02
	May 21–30	31.33	21.33	–	26.33
Mv Maraton	Apr. 1–10	19.33	12.00	25.53	18.95
	Apr. 21–30	21.33	13.33	23.80	19.49
	May 1–10	34.67	13.33	28.87	25.62
	May 21–30	32.00	20.00	–	26.00
Szegedi 428	Apr. 1–10	19.33	11.33	24.13	18.26
	Apr. 21–30	26.67	11.33	24.67	20.89
	May 1–10	32.00	11.33	32.47	25.27
	May 21–30	32.00	20.00	–	26.00
Mv Vilma	Apr. 1–10	21.33	13.33	29.27	21.31
	Apr. 21–30	24.67	14.00	26.80	21.82
	May 1–10	32.67	14.67	35.60	27.65
	May 21–30	34.67	22.00	–	28.34

In 2004, early and very early hybrids achieved their highest yields when sown early or at the optimal sowing date, while the highest yield for FAO 400–500 hybrids was obtained when they were sown on 13 May, though this late sowing date resulted in a 6–9% higher moisture content at harvest. Averaged over three years, the yields of early and late hybrids were 6.12–7.62 t/ha and 7.32–9.91 t/ha, respectively.

The grain moisture content at harvest (Table 6) can be reduced considerably by earlier sowing. Hybrids with good chilling resistance at germination are suitable for early sowing.

Table 6
Moisture content (%) at harvest, averaged over hybrids

Sowing date	Moisture content
Sowing date I (9–17 April)	17.99
Sowing date II (24–26 April)	19.68
Sowing date III (7–8 May)	24.18
Sowing date IV (13–17 May)	24.67

Maize yields and yield stability can be enhanced by growing early, high-yielding (FAO 300) hybrids and by sowing 10–15 days earlier than the date (25–26 April) previously considered to be optimal.

Plant density has a decisive effect on yield. With increasing plant density, the yield per plant (ear size) decreases, but the yield per unit area increases until the optimal number of plants/ha is reached.

Optimal plant density is greatly influenced by the genetic characteristics and vegetation period of the hybrid, the growing site, the year effect and the nutrient and water supplies.

There are great differences between the hybrids in their responses to increasing plant density (Table 7).

In 2002, the maximum yield was obtained at a plant density of 75 thousand plants/ha for hybrids Mv SC 277, Debreceni 377 and Mv Maraton, and at 45 thousand plants/ha for Szegedi 428 and Mv Vilma (FAO 400–500). In the dry year of 2003, the yields ranged from 4.15–7.46 t/ha and the correlation between plant number/ha and yield was weaker.

Table 7
Effect of plant density on the yield of maize (t ha^{-1})

Hybrid	Thousand plants/ha	Yield t/ha			Average
		2002	2003	2004	
Mv SC 277	45	7.59	5.10	9.25	7.18
	60	8.40	5.00	10.17	7.86
	75	9.20	5.63	11.20	8.68
	90	8.70	6.09	11.41	8.73
Borbála	45	7.40	4.15	8.31	6.62
	60	8.21	4.84	9.97	7.67
	75	8.20	4.88	10.48	7.85
	90	8.91	5.61	11.37	8.63
Debreceni 377	45	7.52	4.72	9.56	7.27
	60	8.04	5.25	10.40	7.90
	75	8.46	7.46	11.73	9.22
	90	8.45	6.30	11.97	8.91
Mv Maraton	45	8.61	5.24	10.75	8.20
	60	8.02	5.33	10.88	8.08
	75	8.77	5.82	11.80	8.80
	90	8.36	6.03	11.01	8.47
Szegedi 428	45	7.39	5.32	9.46	7.39
	60	7.03	5.66	9.44	7.38
	75	6.94	6.27	10.38	7.86
	90	6.52	6.47	10.69	7.89
Mv Vilma	45	8.25	5.07	9.67	7.66
	60	7.89	5.73	10.64	8.09
	75	7.58	5.98	10.79	8.12
	90	6.81	6.03	12.06	8.30
$LSD_{5\%}$		1.49	0.20	1.18	

In 2004, the yields were 40–50% higher than in 2003 and the correlation between plant density and yield was close. All the hybrids, with the exception of Mv Maraton, reached the maximum yield of 10.69–12.06 t/ha at a plant density of 90 thousand plants/ha, but the yield increments of 0.31–1.27 t/ha at a plant density above 75 thousand plants/ha were not significant ($LSD_{5\%}$=1.51).

In a favourable year, the hybrids produce maximum yield at a higher plant density, since the decrease in the individual plant yield due to increasing plant density is smaller than in a dry year. The optimum plant density interval should be determined, as well as the optimal plant density, and low or medium values should be chosen, because the number of infertile plants increases at high plant density in a dry year, with a consequent reduction in yield.

The optimal plant densities for the hybrids, averaged over three years, were:

Mv SC 277, Debreceni 377 and Mv Maraton: 75 thousand plants/ha
Szegedi 428, Mv Vilma: 60 thousand plants/ha
Borbála: 90 thousand plants/ha

The hybrids can be classified in four groups according to their responses to increasing plant density:

1. Hybrids responding well to higher density and having a wide optimal plant number interval: Mv SC 277, Borbála
2. Prolific hybrids with high individual plant yield, giving high yields and yield stability even at a relatively low plant density: Mv Maraton
3. Hybrids with a flexible ear type, with increasing ear size in favourable years (high plant density is not recommended): Szegedi SC 428, Debreceni 377
4. Hybrids that respond sensitively to increasing plant density: Mv Vilma. In an unfavourable year, higher plant density results in a considerably lower yield.

The frequency of dry years has increased recently and lower nutrient rates are applied, so the relatively high plant densities used in the 1970s and 1980s are no longer recommended.

In hybrid-specific technologies, the NPK fertilizer rates, the sowing date and the plant density must be adapted to the given hybrid, the level of inputs must be ensured and positive interactions between the ecological, biological and agronomic factors should be exploited.

Acknowledgements

Thanks are due to the team of the OM 4/0008/2002 maize consortium for providing the conditions for the research and for their help during the work, and to Prof. János Nagy for his professional guidance and support.

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Acta Agronomica Hungarica, 53(1), pp. 71–79 (2005)

STRATEGIES FOR CONTROLLING WESTERN CORN ROOTWORM (*DIABROTICA VIRGIFERA VIRGIFERA*)

E. SZÉLL[1], I. ZSELLÉR[2], G. RIPKA[3], J. KISS[4] and G. PRINCZINGER[5]

[1]CEREAL RESEARCH NON-PROFIT CO., SZEGED, HUNGARY
[2]PLANT AND SOIL PROTECTION SERVICE OF CSONGRÁD COUNTY, HÓDMEZŐVÁSÁRHELY, HUNGARY
[3]PLANT AND SOIL PROTECTION SERVICE CENTRE, BUDAPEST, HUNGARY
[4]SZENT ISTVÁN UNIVERSITY, GÖDÖLLŐ, HUNGARY
[5]MINISTRY OF AGRICULTURE AND RURAL DEVELOPMENT, BUDAPEST, HUNGARY

Received: 18 March, 2005; accepted: 27 April, 2005

Western corn rootworm (WCR) first appeared in Europe near Belgrade airport in 1992. The first adult of this species was found near Mórahalom, in the southern part of Hungary, on 30th June 1995. Small-plot trials were started in Szeged (Cereal Research Non-Profit Co.) and large-scale trials in Mezőhegyes (Ménesbirtok Ltd.) in 1996 to elaborate strategies to control this insect pest. To make the work more efficient, eight experts working at different institutions formed a team. At the same time, monitoring was begun on when and where the insect appeared and in what numbers. The experiments were adapted to the life cycle of the species. WCR has one generation per year, laying eggs that overwinter. Larvae begin to hatch in late May to mid-June. Adults emerge by mid-July and survive till early October. They lay eggs in abundance from mid-July to late August.

Trials were conducted to control WCR both with and without chemicals. For adult control pesticides were sprayed from an aircraft or using a field sprayer. Larval control involved seed treatment with insecticide or the use of soil insecticide at planting or at cultivation.

Key words: *Diabrotica virgifera virgifera*, adult, egg, larva, crop rotation, trapping, seed treatment, soil insecticide

Introduction

Since 1992, when western corn rootworm (WCR) was first observed near Belgrade (then Yugoslavia, now Serbia and Montenegro), the affected area has increased to 5,695 ha in 1997, 45,713 ha in 1998, 30,000 ha in 1999 and 50,000 ha in 2000 (Bača and Sivcev, 2001). These data prove three important facts:

- The number of adults increases rapidly after the first appearance, and their larvae can soon cause economic damage by feeding on maize roots;
- Adults can spread rapidly;
- The damage caused by WCR is greatly influenced by the season. It can be said that, if the weather is favourable for maize plants, it is favourable for WCR, too. It is worth mentioning here that all the cultivation procedures, except crop rotation, carried out to increase the average yield of maize, enhance the reproduction of WCR as well.

0238–0161/$ 20.00

WCR appeared in Hungary in 1995, and root lodging due to larval damage could be observed in 1999. The weather was rather unfavourable both for maize and WCR in 2003. In that year the season was dry and hot, the maize plants matured very early, and the adults reduced their egg production due to lack of food. The texture of the dry soil was also unfavourable for egg-laying. As a result, WCR caused far less damage nationwide in 2004 than in 2003. By contrast, the weather in 2004 was favourable from both aspects, as there was a very high yield and the conditions for egg-laying were ideal. More serious larval damage is thus expected in 2005 than in 2004.

The larvae of WCR cause damage first by feeding on the roots and then by tunnelling into the roots. Larval damage reduces the nutrient and water uptake of the plants and leads to plant lodging. Cases have been registered in Hungary where 100% of the plant stand lodged on a given area and no maize could be harvested.

Root damage is rated on the IOWA 1–6 scale, where the value 6 represents the worst damage. The economic threshold is 3.0–3.5 on the IOWA scale. Adults generally feed on pollen and trim the silks, but in the absence of these, they strip the green tissue of the leaves. They may even damage the grains at the milky and waxy stages. Adults can cause severe damage on areas sown for seed production or sweet corn production. Ears damaged by the beetles become susceptible to *Fusarium* infection. If the maize plants mature early, the adults may feed on green weeds.

It is important to note that WCR is not spread by maize seed (Hatala-Zsellér and Ripka, 2001). Larvae can only migrate a very short distance (10–40 cm) in the soil. Adults can fly and may migrate 30–40 km/year. However, they are likely to get much farther if trapped in vehicles.

WCR samples can be caught in pheromone traps, which attract male adults from a considerable distance, or using bright yellow sticky sheets which attract the beetles visually.

The control of WCR is based on prediction: if 0.5–0.7 adults are found per plant, WCR must be controlled next spring. If there are two or more larvae on the roots of the maize plant, economic damage is likely to occur.

When elaborating strategies to control WCR, experiences gained in Yugoslavia and by the US expert, Dr. Richard C. Edwards, were made use of.

Crop rotation is a major strategy for controlling WCR without chemicals. Wheat is the best pre-crop. Soybean is not a good pre-crop, because adults are also able to feed on soybean plants. It is very important to keep the field free from volunteer plants by soil cultivation, because adults may feed on these plants when they emerge.

Irrigation can have the following impacts: on the one hand, it improves root regeneration, thus reducing damage; on the other hand, egg-laying becomes easier on wet soil, so it also enhances the spread of WCR.

The importance of investigations on WCR in Hungary and of the development of a control strategy is confirmed by the number of publications on this topic: around 35 papers in Hungarian and 19 summaries of conferences have been published on the biology, life cycle, spread and control strategies of WCR (Gyakorlati Agrofórum 2001; 2002).

The number of papers published in 2004 and 2005 indicate that controlling WCR has become a central problem. The manufacturers of herbicides and insecticides, and the Hungarian authorities have agreed to apply the results of WCR research in practical maize production within the shortest possible time.

Materials and methods

The small and medium plot trials were set up with the co-operation of experts dealing with various research areas in different institutes.

First the resistance of different hybrids was tested. The machines needed for the field trials were assembled at the Cereal Research Non-Profit Co. A granulate spreading adapter and a fluid sprinkling adapter were fitted to the seed drill and the cultivator.

Maize is no longer grown in a monoculture at the research station in order to reduce the damage caused by WCR, except for one experimental block, where maize has been grown in a monoculture since 1979. Consequently, it is possible to compare the efficiency of crop rotation to that of a maize monoculture each year.

Three types of chemical control were tested:

Areawide pest management for adult control, involving spraying from an aircraft or a field sprayer.
Larval control by seed treatment with insecticide, tested at two planting dates (23rd April and 15th May).
Soil insecticide at planting or cultivation.

When treatment was applied at planting, a granulate spreading adapter or a fluid sprinkling adapter was fitted to the seed drill. Insecticide in granulate or fluid form was placed in-furrow next to the seed. The compacting wheel of the seed drill closed the soil above both the seed and the insecticide.

When treatment was applied at cultivation, a granulate spreading adapter or a fluid sprinkling adapter was fitted to the cultivator. The insecticide was placed in the soil 7–10 cm from the seed, and a special covering device closed the soil. Soil insecticide was used at the recommended rate per hectare both at planting and at cultivation, so one dose was placed in-furrow with the seed, and another "extra dose" was placed close to the seed.

Evaluation

To estimate root damage, the plants were dug up and the roots were washed free of soil. The roots were then scored according to the IOWA scale, where 1 = no damage and 6 = severe damage (larvae have chewed 95–99% of the root). Root lodging was estimated by calculating the ratio of lodged plants. Yields were recorded, and the data were analysed using variance analysis.

The results reflected seasonal effects, so the most important meteorological data for 2003 and 2004 are summarized in Table 1.

The weather was drier and warmer in 2003 than the long-term average. The year 2004, however, was more favourable for maize than the average.

Table 1
Comparison of meteorological data in the Szeged area in 2003 and 2004

Period	Precipitation, mm		Monthly average temperature °C		Number of extremely hot and hot days	
	2003	2004	2003	2004	2003	2004
Winter half-year	185	248	11.0	12.3	–	–
April	21	89	20.9	15.8	–	–
May	21	49	24.3	20.3	9	–
June	28	64	24.5	23.4	13	1
July	49	152	24.0	22.8	12	14
August	5	25	–	–	17	5
Total	124	379	–	–	51	20
Winter half-year + maturity season	309	627	–	–	–	–

Results

Control of WCR without chemicals

Resistance of maize hybrids to larval damage caused by WRC

The choice of resistant maize hybrids could be the first step in strategies to control WCR. The resistance of hybrids can only be tested on areas where WCR occurs. As far as we know, no damage by WCR larvae has yet been recorded on sandy soils with low or medium humus content (owing to the quartz crystal content).

The resistance of 18 Szeged maize hybrids grown in a monoculture was tested in 2003, and that of 15 in 2004 (Tables 2 and 3).

Before analysing the data, mention should be made of the seasonal effect. The year 2003 was dry and hot, and very early (FAO 200) hybrids were found to lodge less than medium or late hybrids (FAO 400–500). The weather was more favourable in 2004 and, averaged over the hybrids, significantly fewer plants were recorded to have lodged (7%) than in 2003 (16%). It was observed, moreover, that fewer (5%) medium and late hybrids (FAO 400–500) lodged compared with the very early (FAO 200) ones (10%). The soil was dry in 2003, and plant lodging was influenced by the centre of gravity of the plant above the soil, in addition to the level of root damage.

In 2004, however, the root system of the plants was able to regenerate rapidly in the wet soil. Hybrids with a longer vegetation period presumably developed larger root volume, so that the larvae caused less damage than in hybrids with a shorter vegetation period. In all probability, the lower level of root lodging in later hybrids compared to earlier ones in 2004 can be ascribed to this fact.

The wide deviation in the root lodging reflects the specific resistance of hybrids to larval damage. Hybrids with only 0–2% lodging were found in both years, and this fact encourages us to believe that it is possible to breed hybrids resistant to larval damage by conventional breeding (without GMO).

Table 2
Susceptibility of maize hybrids with different maturity and genotype to damage by WCR larvae in monoculture (Újszeged, 2003–2004)

Year	Maturity group	No. of hybrids tested	Root lodging %		
			Average	Extreme values	
				highest	lowest
2003					
	FAO 200	6	6	25	0
	FAO 300	6	19	30	1
	FAO 400-500	6	22	35	2
		Average	16	30	1
2004					
	FAO 200	3	10	14	8
	FAO 300	6	7	23	0
	FAO 400-500	6	5	13	0
		Average	7	17	3

Table 3
Root lodging caused by WCR larval damage as a function of hybrids and production technology (Újszeged, 2004)

Hybrid code number	Root lodging (%) as a function of production technology	
	Monoculture	Crop rotation
1	8	0
2	9	0
3	14	0
4	0	0
5	0	0
6	6	0
7	9	0
8	23	0
9	3	0
10	2	0
11	1	0
12	6	0
13	0	0
14	13	3
15	6	0
Average	7	0

WCR control by means of crop rotation

The data in Table 3 demonstrate that while root lodging averaged 7% for 15 maize hybrids grown in a monoculture, it was 0% in crop rotation in 2004. Even a hybrid that exhibited 23% root lodging in a maize monoculture did not lodge in the case of crop rotation.

The data in Table 4 confirm the advantage of crop rotation. If maize was grown in a monoculture for 3 years instead of 2 years, root lodging increased significantly with a parallel reduction in yield.

Table 4
Root lodging caused by WCR larval damage and grain yield as a function of the number of years of continuous maize (Újszeged, 2003)

No. of years of continuous maize	Root lodging		Grain yield	
	%	D	t/ha	%
2nd year	4	0	8.8	100
3rd year	21	17	8.3	94
$LSD_{5\%}$	4		1.0	11

WCR control with insecticide

Areawide pest management for controlling adults

Areawide pest management was carried out several days before egg-laying. Sampling with traps proved that approx. 70–80% of the adults were killed, so the number of larvae in the following year was reduced. The efficiency of the method may be questionable, however, if adults fly to the area from untreated fields a few days later. Areawide pest management is most effective if it is done on a large area.

Medium and large-scale trials were conducted to improve the efficiency of areawide pest management and to make it environmentally friendly (Edwards et al., 1999; Petró et al., 1998).

Areawide pest management was carried out from an aircraft on large fields, and with a field sprayer on medium plots. The trials proved that this type of control could be applied both on small farms and on large areas.

Control of larval damage

The basic principle of this management strategy is for the insecticide concentration in the soil to be sufficient to control the larvae of WCR. It has to be available at the proper place in the soil, near the roots of the maize plants, and at the proper time, from late May to mid-June.

One solution seemed to be seed treatment with insecticide and the other the development of a specific technique for applying soil insecticide.

Seed treatment to control larvae

This management strategy was based on the fact that maize can be protected from the most common insect pests, from planting to the 35-leaf stage, by treatment with insecticide. However, when the results of the trials were analysed, especially for yield, in 2003 and 2004, seed treatment with insecticide was found to be the least efficient way of controlling WCR (Fig. 1).

Seed treatment with insecticide did not reduce the root damage rate according to the IOWA 1–6 scale compared to the check in 2003, and there was only an improvement of 0.5 in 2004 when planting was carried out in mid-April. Moreover, this type of treatment was less efficient in reducing root lodging in 2003. (Root lodging: check = 16%; seed treatment with insecticide = 7%; soil insecticide at planting = 1%.) Root lodging was negligible in 2004.

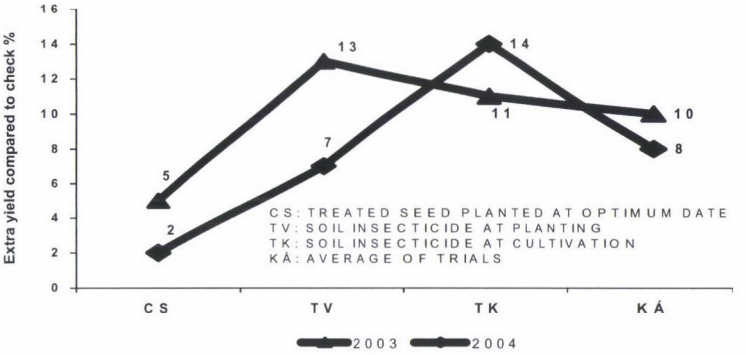

Fig. 1. Comparison of different insecticide application methods for controlling WCR larvae in two years, Újszeged, 2003–2004

The results confirmed that late planting could improve the larva-controlling effect of chemical treatment, as the insecticide was applied nearer to the period of larval hatch. Nevertheless, late planting reduced yield, and the yield loss could not be counterbalanced by chemical treatment (Table 5). In summary it can be stated that the yield after chemical seed treatment was 0.6 t lower after late planting (5th May) than the yield of untreated plants sown at the optimum date (23rd April).

Soil insecticide at planting to control WCR larvae

The treatment resulted in a 10% yield increase averaged over two years (2003 = 13%, 2004 = 7%), which exceeded the effect of seed treatment with insecticide by 6.5% (Fig. 1). Root damage was 0.7 lower on the IOWA scale compared to the check in 2003, and root lodging decreased from 16% to 1%. In 2004 these figures were 0.4 for root damage, while root lodging decreased from 4.4% to 0.5%. The seasonal effect influenced the results: damage was more severe in 2003 than in 2004 due to the unfavourable weather conditions.

Table 5

Joint effect of planting date and seed treatment with insecticide on the grain yield of maize in a WCR management trial (Újszeged, 2004)

Planting date	Chemical treatment	Grain yield		
		t/ha	D_1	D_2
20th April	control	13.2	0.0	0.0
	seed treatment	13.5	0.3	0.0
15th May	control	11.7	0.0	–1.5
	seed treatment	12.6	0.9	–0.9
$LSD_{5\%}$ for each difference			0.7	

D_1 difference within each planting date; D_2: difference between planting dates

Soil insecticide at cultivation to control larvae

The result of this management strategy was a 12.5% yield increase averaged over two years (2003 = 11%, 2004 = 14%) (Fig. 1). Root damage diminished by a value of 0.3 in 2003 and 0.5 in 2004 compared to the check. Root lodging diminished from 16% to 5.0% in 2003, and from 1.6% to 0.6% in 2004.

After analysing the yields in the trials with soil insecticide the following conclusions were drawn: if there was less precipitation than average in spring, soil insecticide applied at planting was more effective, but if there was more precipitation than average in spring, soil insecticide applied at cultivation was more efficient. On the basis of the results it can be stated that the moisture content of the soil is an essential factor in judging the efficiency of soil insecticides.

Discussion

Western corn rootworm (*Diabrotica virgifera virgifera*), an insect pest capable of causing extensive losses in maize, was first discovered in Europe in 1992, and in Hungary in 1995. Larval damage may be the most serious, because the larvae feed on the roots, preventing the plants from taking up water and nutrients. The plants may also lodge, with dramatic yield loss as a consequence.

Based on the results, there appear to be two possible strategies to control WCR.

Methods without chemicals include the choice of hybrids tolerant of WCR damage and crop rotation, both of which may lead to good results.

Insecticides can be used in two ways to control WCR, one being areawide pest management to control adults. This treatment gives the best results if a large area is sprayed rather than small plots.

The other method is to control larvae, either through seed treatment with insecticide or using soil insecticide. Seed treatment with insecticide was less efficient than soil insecticide at planting or at cultivation. Soil insecticide was more effective at planting in dry weather (2003), and at cultivation under wet conditions.

Acknowledgements

The authors would like to express their thanks to the Ministry of Education for the funds provided by the National Research and Development Programs (subprogram: Research in Agrarian Economy and Biotechnology, No. 4/0008/2002); to the Centre of Agricultural Sciences, University of Debrecen, for heading the maize consortium and for organizing the project work; to the partner institutions, for allowing their colleagues to participate in the co-operation; to colleagues who allowed their experience (in written or oral form) to be used in this work; to the Cereal Research Non-Profit Company for allowing the trials to be conducted since 1996; to the colleagues who contributed to the project, and to Mrs. L. Buza for her administrative assistance.

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Acta Agronomica Hungarica, 53(1), pp. 81–87 (2005)

COLD TOLERANCE OF INBRED LINES AND SISTER LINE CROSSES OF MAIZE WITH DIFFERENT GENETIC BACKGROUNDS

T. SZUNDY, I. PÓK and C. L. MARTON

AGRICULTURAL RESEARCH INSTITUTE OF THE HUNGARIAN ACADEMY OF SCIENCES, MARTONVÁSÁR, HUNGARY

Received: 10 April, 2005; accepted: 20 May, 2005

An improvement in the early spring cold tolerance of maize would allow it to be grown in more northern areas with a cooler climate, while on traditional maize-growing areas the profitability of maize production could be improved by earlier sowing, leading to a reduction in transportation and drying costs.

The cold tolerance of crosses between inbred lines and sister line crosses belonging to three related groups that combine well with each other (BSSS, Iodent, Lancaster) was tested in the Martonvásár phytotron.

The results confirmed those of earlier experiments and led to the following new conclusions:

– the average emergence time of the tested Iodent inbred lines was longer than that of the BSSS and Lancaster groups,

– all three groups contained inbred lines with significantly earlier emergence than the others,

– the average emergence percentage and individual shoot dry matter production in the Iodent group were also lower than in the other two groups,

– a close negative correlation ($r = -0.70$) was found between the number of days to emergence and the individual dry shoot mass.

The results were used to select inbred lines and sister line crosses with various genetic backgrounds that could be used in crosses aimed at improving the resistance of hybrids to cold stress in early spring.

Key words: cold tolerance, maize inbred lines

Introduction

Maize yield averages are substantially influenced by resistance to abiotic stress, including chilling in early spring. The success of breeding for improvements in maize cold tolerance has resulted in the expansion of maize production areas into more northerly regions.

Experimental results and practical experience have shown that earlier sowing generally leads to earlier maturity and lower grain moisture content at harvest (Kovács, 1958; Pendleton, 1965). One of the most important preconditions for early sowing is an improvement in cold tolerance at germination.

The germination of maize seeds in cold, damp soil has been investigated by many authors (Meyers, 1924; Tatum, 1942; Tatum and Zuber, 1943; Neal, 1950; Pinell, 1949; Koehler, 1954; Hoppe, 1949; 1955; 1966, etc.). These

0238–0161/$ 20.00

authors found that germination in cold soil was greatly influenced by the age of the seed and the conditions under which the seed was produced (early frost, maturity at harvest, method of harvesting, shelling, seed cleaning and grading). It is therefore advisable to use seed produced in the same year, at the same location, under the same conditions in cold tolerance tests.

Neal (1950) and Rinke (1954) drew attention to the fact that the female parent had a decisive role in the inheritance of cold tolerance, while Neptune (1953) noted that the "cold test" values deteriorated as the degree of inbreeding increased.

Another important component in maize cold tolerance is the ability of seedlings to adapt to the cool, wet periods often occurring in spring. The cold tolerance of seedlings was studied by Gupta and Kovács (1974) in "cold wave" experiments, which revealed differences in the genetic background of cold tolerance at germination and in the seedling stage.

The cold tolerance of maize inbred lines was investigated by Mock and Neil (1979), who reported a significant correlation between dry seedling mass and the yield.

The Martonvásár phytotron provides an excellent opportunity for the study of cold tolerance. Herczegh (1978) elaborated a new method for the analysis of maize cold tolerance in the phytotron and found that inbred lines belonging to the BSSS group had better cold tolerance than those in the Lancaster group.

Szundy and Kovács (1981a, b) studied the cold tolerance of maize genotypes and hybrids with different levels of heterozygosity in the phytotron and revealed that populations with 50 or 100 % heterozygosity emerged faster and more reliably than those with 0 or 25 % heterozygosity. Previous knowledge on maize cold tolerance was expanded by these authors, since it was found that an increase in the level of heterozygosity of the female parent resulted in an improvement in cold tolerance.

When studying the cold tolerance of maize S_2 families, Quang and Szundy (1989) concluded that there was sufficient variability between the S_2 families to allow successful selection. A significant negative correlation was found between the emergence date and the emergence percentage, and between the emergence date and the individual fresh and dry shoot mass.

Marton et al. (1990) studied maize cold tolerance in the gradient chamber of the phytotron. Further results of maize cold tolerance studies in the Martonvásár phytotron were summarised by Marton et al. (1997) and Szundy (1997). It was found that the agronomic value of maize hybrids could be maximised by crossing elite inbred lines with different genetic backgrounds.

In the course of the present research the aim was to determine the cold tolerance of maize inbred lines belonging to related groups that combine well with each other, and to discover whether there was sufficient difference between the cold tolerance levels of inbred lines within the individual related groups to allow satisfactory crossing partners to be identified.

Materials and methods

Tests were made on the cold tolerance of four maize inbred lines from each of the BSSS, Iodent and Lancaster groups, in the third or fourth cycles, and of three sister line crosses, one representing each group. The following cold tolerance traits were analysed:

– number of days to emergence,
– percentage emergence, as a percentage of the control,
– dry shoot mass per plant.

Wooden boxes measuring 40 × 60 cm were used in the experiments. Seed of 20 genotypes were sown in each box, with 3 cm plant and row spacings and 10 seeds per replication. The seed was produced in the institute nursery in 2002. The boxes were filled with standard maize soil stored in the phytotron, and were saturated with tap water to 70% field water capacity. In order to avoid the caking of the soil due to irrigation, the boxes were covered with a 1 cm layer of sand. The mass of the boxes was measured each day and the quantity of water evaporated was replaced.

The number of emerged plants was counted daily from the beginning of emergence. The number of days to emergence was calculated using a weighted mean.

On the 30th day after sowing the plants were cut off at the soil surface and the dry matter mass per plant was recorded after drying the shoots to constant weight in a drying oven.

The seeds of the genotypes included in the experiment were also sown under optimum conditions in order to screen the tests for any differences in germination and emergence dates.

The data were evaluated using analysis of variance.

The following programme was applied to test cold tolerance:

Temperature	+8°C		10 days
	+13.5°C		20 days
Relative humidity	day	75%	
	night	70%	
Illumination	20 klx in three stages		
		6.00–16.00	1/3
		7.00–17.00	2/3
		8.00–18.00	3/3

The climate programme representing optimum conditions was as follows:

Programme duration		14 days
Temperature	day	+24°C
	night	+18°C
Relative humidity	day	75%
	night	70%
Illumination	as in the cold programme	

In the chamber providing optimum conditions, emergence began 4 days after sowing and was completed on the 5th day. There was no significant difference in emergence percentage between the genotypes, so it proved unnecessary to compare the data with the control.

Results and discussion

The data on the cold tolerance traits of the maize inbred lines are presented for each related group in Table 1.

Averaged over the related groups, the earliest emergence was observed for the BSSS group. The Lancaster group emerged significantly later, and the Iodent group significantly later than both.

Among the inbred lines in the BSSS group, genotypes BS 78 and BS 31 emerged latest, while BS 33 and BS 47 emerged significantly earlier than these.

Table 1
Cold tolerance of maize inbred lines

Genotype	Days to emergence	Emergence (%)	Dry matter per plant (g)
BS 33	16.78	87.5	43.17
BS 47	16.91	75.0	55.35
BS 31	18.41	77.5	27.18
BS 78	19.00	97.5	18.96
BSSS mean	17.78	84.4	36.17
Io 41	17.03	82.5	38.81
Io 26	18.47	62.5	28.49
Io 58	18.26	85.0	26.28
Io 46	21.46	65.0	20.91
Iodent mean	18.81	73.8	28.62
La 06	18.05	100.0	33.30
La 17	17.94	87.5	36.31
La 18	16.91	77.5	30.79
La 29	20.15	82.5	25.50
Lancaster mean	18.26	86.9	31.48
Inbred line mean	18.28	81.70	32.09
$LSD_{5\%}$ lines	1.17	17.84	8.71
$LSD_{5\%}$ means	0.35	6.26	2.17

In the Iodent group, Io 46 emerged significantly later and Io 41 earlier than the other two genotypes in the group (Io 26 and Io 58).

Among the maize inbred lines in the Lancaster group, La 18 emerged significantly earlier than the other genotypes, while La 29 emerged significantly later than the genotypes La 17 and La 06.

The data on the mean emergence date of the groups confirm findings in the literature stating that genotypes from the BSSS groups generally emerge earlier in cold, wet soil than the Lancaster type. The present data provide the extra information that the mean emergence date of the Iodent inbred lines examined was later than either of the other groups.

It was clear from the results, however, that all the groups included lines that emerged significantly earlier or later than the average, allowing inbred lines with good combining ability and better cold tolerance to be chosen from each group. This could lead not only to improvements in the reliability of hybrid maize seed production, but also in the cold tolerance of the F_1 seed.

No significant difference was found between the mean emergence percentages of the BSSS and Lancaster inbred lines, but that of the Iodent group was poorer than either.

Within the BSSS group the best emergence percentage was exhibited by BS 78 and BS 33, while the emergence of BS 47 and BS 31 was significantly poorer than that of BS 78.

Among the inbred lines belonging to the Iodent group, Io 41 and Io 58 had the highest emergence percentages, which were significantly better than those of Io 26 and Io 46.

The best emergence in the Lancaster group was observed for La 06, but only that of La 18 was significantly worse.

The mean dry shoot mass per plant in the various groups indicated that BSSS maize inbred lines had significantly greater dry matter mass per plant under cold conditions than the other two groups. The mean for the Iodent group was significantly lower than that of either the BSSS or the Lancaster group.

Among the inbred lines in the BSSS group the greatest shoot dry mass was recorded for BS 47, while that of BS 31 and BS 78 was significantly smaller than that of BS 47 and BS 33.

In the Iodent group, the individual dry mass production of Io 41 was significantly greater than that of the inbred lines Io 26, Io 58 and Io 46. There were no significant differences between the three latter genotypes.

The lowest dry matter mass per plant in the Lancaster group was measured for La 29, while the difference in this parameter for La 17, La 06 and La 18 was not significant.

A close significant negative correlation ($r = -0.70$) was found between the shoot dry matter production per plant and the emergence date. The shorter the period to emergence, the larger the shoot dry mass per plant.

As in the case of the emergence date, an analysis of the data on the dry shoot mass per plant and the emergence percentage led to the conclusion that differences within the related groups were sufficient to allow better crossing partners to be selected.

The cold tolerance traits of the sister line crosses from each related group are presented in Table 2. The data confirm the conclusions drawn concerning the cold tolerance traits of maize inbred lines, with the exception of the emergence percentage. No significant difference was found between the emergence percentages of sister line crosses in different related groups. It is interesting to note that the data indicated earlier emergence, better emergence percentage and higher dry shoot mass per plant than the mean for the inbred lines.

Table 2
Cold tolerance of maize sister line crosses

Genotype	Related group	Days to emergence	Emergence (%)	Dry matter per plant (g)
BS 33 × BS 47	BSSS	15.97	97.5	56.2
Io 26 × Io 41	Iodent	18.45	95.0	33.73
La 06 × La 17	Lancaster	18.74	95.0	42.01
Mean		17.77	94.6	43.43
$LSD_{5\%}$		1.17	NS	8.71

These results supplement the data of Neptune (1953), since it appears that although the "cold test" values deteriorate with the degree of inbreeding, an increase in the level of heterozygosity leads to an improvement in cold tolerance traits.

The research results were used to develop maize hybrids expected to have more reliable seed production and better cold tolerance than that of hybrids developed by crossing parents with unknown cold tolerance traits.

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Acta Agronomica Hungarica, 53(1), pp. 89–98 (2005)

CHANGES IN AGRONOMIC TRAITS AFFECTED BY PHOTOPERIOD AND VERNALIZATION IN A GROUP OF WILD BARLEY ACCESSIONS (*HORDEUM VULGARE* SSP. *SPONTANEUM*) AND BARLEY CULTIVARS (*HORDEUM VULGARE* L.)

I. KARSAI, K. MÉSZÁROS, L. LÁNG and Z. BEDŐ

AGRICULTURAL RESEARCH INSTITUTE OF THE HUNGARIAN ACADEMY OF SCIENCES, MARTONVÁSÁR, HUNGARY

Received: 3 March, 2005; accepted: 2 May, 2005

The effect of vernalization response and photoperiod sensitivity on reproductive fitness and agronomic traits was examined in a group of 16 *H. spontaneum* accessions and 8 *H. vulgare* cultivars in controlled environments. The whole range of plant developmental and agronomic traits was determined by vernalization. The reproductive fitness was severely impaired when the vernalization requirements of the plants were not saturated. Variation in the magnitude of vernalization response significantly correlated with several traits. A larger decrease in reproductive tiller number, average seed number and consequently final grain yield was more characteristic of accessions with a greater vernalization response. When the vernalization requirement was met, long photoperiod enhanced the fitness of the plants and resulted in larger yield and yield components, irrespective of the genotype, while short photoperiod acted as a limiting factor for all these traits. There was, however, a difference in the reaction type of wild and cultivated genotypes due to their different plant strategies.

Key words: *Hordeum spontaneum*, vernalization, photoperiod, yield components

Introduction

In cereals the flowering time is one of the basic factors influencing their adaptability to different environments. Three classes of genes controlling heading date are known in cereals, all of which influence the rate of plant development and/or control the change from vegetative to reproductive growth. Low temperature influences the genes of vernalization response (Takahashi and Yasuda, 1971; Danyluk et al., 2003; Yan et al., 2003; 2004), changes in daylength control the loci of photoperiod response (Laurie et al., 1994; 1995), while other sets of genes determine flowering time largely independently of these environmental factors. The latter have been termed earliness *per se*, or developmental rate genes (Laurie et al., 1995). In addition to controlling flowering time, these component traits play a significant role in influencing plant developmental and yield component traits by contributing to the determination of the length of various plant developmental phases such as the length of the vegetative period and inflorescence initiation (Laurie et al., 1995; Worland, 1996; Karsai et al., 1999).

0238–0161/$ 20.00

Hordeum vulgare ssp. *spontaneum* is known to be one of the progenitors of all cultivated barley varieties (Nevo et al., 1984). The vernalization response, photoperiod sensitivity and relative earliness were investigated in a sample of *Hordeum spontaneum* accessions (Karsai et al., 2004) and were found to represent special combinations of adaptational traits not present in cultivated barleys. Accessions originating from the Fertile Crescent were of winter growth type and possessed photoperiod sensitivity, but most of them were relatively early flowering, which is not characteristic of the majority of cultivated winter barleys (Karsai et al., 2001). All the winter *spontaneum* accessions, however, possessed a significantly lower vernalization response than the cultivated winter barleys, suggesting that during domestication and breeding for adaptation to more extreme winter environments than those of much of the Fertile Crescent, additional "winter habit" alleles (structural and/or regulatory) have been selected (Kato et al., 1997; Iwaki et al., 2001). The two accessions from Nepal and Tibet represented a typical spring growth habit, which was accompanied by an extremely strong sensitivity to short photoperiods, not apparent in cultivated spring barleys (Karsai et al., 2001).

Based on the difference found in adaptational traits between *spontaneum* accessions and barley cultivars, the major aim was to examine the effect of photoperiod and vernalization on the fitness and agronomic traits of this sample of wild and cultivated barley genotypes.

Materials and methods

Sixteen *Hordeum spontaneum* accessions (referred to as *spontaneum*) and eight barley varieties (*Hordeum vulgare* L.; referred to as cultivars) were included in the experiment to evaluate the effect of photoperiod and vernalization on plant developmental and agronomic traits. Complete information on these *spontaneums* and cultivars was published by Karsai et al. (2004). Fourteen of the *spontaneum* accessions originated from the Fertile Crescent (Hvs1 from the Caspian Sea; Hvs4–Hvs9, Hvs11, Hvs12, Hvs15, Hvs18, Hvs19 from Israel and Hvs16, Hvs17 from Iran) and two from the Himalayan region (Hvs2 from Nepal and Hvs3 from Tibet), all of them having brittle rachis. Among the barley cultivars there was one two-rowed spring type (Harrington), one six-rowed facultative type (88Ab536), five six-rowed winter types (Opale, Pirate, Etrusco, Strider and Kold), and one two-rowed winter type (Arda) (Tuberosa et al., 1997; Karsai et al., 2004).

These 24 genotypes were phenotyped in a series of controlled-environment tests involving combinations of vernalization and photoperiod in CONVIRON PGR-15 growth chambers. The vernalization treatment consisted of 6 weeks of hardening seedlings at 3°C with an 8 h light/16h dark photoperiod regime. Photoperiod treatments, consisting of 8, 10, 12, 14, 16, 18 and 24 h light regimes per 24 h period, were carried out in the phytotron facilities of the Agricultural Research Institute of the Hungarian Academy of Sciences (Martonvásár, Hungary) as described by Karsai et al. (2004). The temperature was kept constant at 18°C day and night in all the treatments. Each genotype was replicated twice. The combined effect of photoperiods and vernalization on heading date was analysed and published by Karsai et al. (2004). Data on plant development and yield components for six photoperiod regimes (8–18 h) in the vernalized treatments and for the 18 h photoperiod regime in the unvernalized treatment are presented here. The following data were recorded for each plant: number of tillers at first node appearance (TIL31), number of tillers at heading (TIL49), number of reproductive tillers bearing spikes (RT), plant height (PH), number of nodes on the main stem (NOD), number of seeds on the main stem (NS), thousand kernel weight of the main stem seeds (TKW), average number of seeds on the side tillers (SNS), and grain yield

of the whole plant (GY). In the case of *spontaneums*, the glumes were removed from the seeds before measuring TKW and GY. The data were processed using the statistical package Excel for Windows. The relationships between vernalization response and developmental and agronomic traits were evaluated using correlation analysis.

Results

Comparing the average values of the two groups, the cultivars headed significantly later than the *spontaneums* in the vernalized treatment with an 18 h photoperiod (Table 1). In addition, the cultivars had shorter plant stature, primarily due to the shorter last internode. The ratio of the last internode to the whole plant height was also smaller in the cultivars than in the *spontaneums* (31 % vs. 43 %). The average node number, the other component of plant height, was similar for the two groups. The average seed numbers on the main and side tillers, the thousand kernel weight, and thus the grain yield were significantly larger in the cultivars than in the *spontaneums*. There were no significant differences in the mean tiller numbers of the two groups measured at various developmental stages.

With the exceptions of seed number on the main tiller and grain yield, the ranges of the different traits measured in the two groups showed various extents of overlapping (Table 1). In the case of heading date the overlapping was due to two *spontaneums* and one cultivar; the two *spontaneums* with spring habit, Hvs2 and Hvs3, headed significantly later (37 and 37.5 days) and the spring barley Opale significantly earlier (27.5 days) than their respective group means. In the case of plant height, three *spontaneums:* Hvs8 (48 cm), Hvs2 (54.5 cm) and Hvs16 (54.5 cm), were shorter than the tallest cultivar (Kold). The overlapping in the seed number on the main stem and side tillers was only due to Hvs3, which had a seed number similar to the two two-rowed cultivars (Arda and Harrington). All the other *spontaneums* produced significantly fewer seeds. In the case of thousand kernel weight, there was again only one *spontaneum* with a value higher than 45 g and only one cultivar with a value lower than 50 g, causing the overlapping of the two groups (Hvs5 and Kold).

Although the *spontaneum* accessions had a smaller vernalization response than the cultivars (Karsai et al., 2004) all their traits were significantly influenced by vernalization treatment, with the exception of plant height (Table 2). In the unvernalized treatment the *spontaneums* headed significantly later, and had a larger number of tillers and more nodes on the main stem. All the other traits showed a dramatic decrease, especially the number of reproductive tillers, thousand-kernel weight and the number of seeds on the side tillers. As a result of these changes the average grain yield in the unvernalized treatment was only 28.0% of that measured in the vernalized treatment. The main tillers of two *spontaneums* (Hvs17 and Hvs18) were completely sterile and there were no ears on the side tillers, so they yielded no grain at all. In five other *spontaneums* (Hvs1, Hvs8, Hvs9, Hvs16 and Hvs19), though the main tillers produced seeds, they either had no ears on the side tillers or these ears were sterile. When the differences between the unvernalized and vernalized treatments of individual

genotypes for a given trait were examined, the correlation between these values and their vernalization responses was found to be significant for several yield components. The number of reproductive tillers, the seed number in the main and side tillers, and the grain yield showed significant negative correlations. As all these traits had smaller values in the unvernalized treatment, this correlation pattern underlined the greater sensitivity of genotypes with greater vernalization response to the lack of vernalization treatment.

Table 1

Average values of different plant developmental and agronomic traits in groups of 16 *Hordeum spontaneum* accessions and of 8 *H. vulgare* cultivars under an 18-hour photoperiod regime in the vernalized treatment

Trait	16 *H. spontaneum* accessions		8 *H. vulgare* cultivars		Difference between averages
	Average	Range	Average	Range	
Heading date (days)	28.8	23.5 – 37.5	36.4	27.5 – 49.0	**
No. of tillers (TIL31)	3.2	1.0 – 6.5	3.9	2.0 – 7.0	ns
No. of tillers (TIL49)	6.8	5.0 – 10.5	7.7	4.0 – 14.0	ns
No. of reproductive tillers	7.6	6.5 – 10.0	5.0	2.0 – 8.0	***
Plant height (cm)	67.0	48.0 – 99.0	51.8	45.0 – 57.0	**
Node number	4.0	3.5 – 4.5	4.5	4.0 – 5.5	*
Length of last internode (cm)	29.0	19.5 – 51.5	16.5	10.5 – 23.0	***
Seed number on main stem	14.6	9.0 – 24.5	41.0	25.0 – 54.5	***
Thousand-kernel weight	32.3	18.3 – 48.0	53.7	43.2 – 61.8	***
Average seed no. on side tillers	12.1	7.9 – 23.0	26.0	18.9 – 45.0	***
Grain yield (g)	2.5	1.0 – 4.3	7.3	5.2 – 10.7	***

*, **, ***: Significant at the P= 0.05, P=0.01 and P=0.001 levels, respectively; ns = non significant

Table 2

Average values of different plant developmental and agronomic traits in groups of 16 *H. spontaneum* accessions in the unvernalized treatment under an 18-hour photoperiod regime and their correlation with the magnitude of vernalization response of the individual accessions

Traits	Unvernalized treatment				Correlation coefficient (r)[3]
	Average	Range	LSD[1]	%[2]	
Heading date (days)	89.8	51.0–121.5	9.3	311.8	1.00
No of tillers (TIL31)	27.2	15.5–52.5	8.2	400.0	–0.01
No. of tillers (TIL49)	3.4	0.0–9.0	4.4	44.7	–0.47+
Plant height (cm)	60.9	43.0–73.5	11.5	90.9	–0.43
Node number	5.7	4.0–7.0	1.4	142.5	0.17
Length of last internode (cm)	14.5	10.0–20.5	5.6	50.0	–0.37
Seed number on main stem	10.2	0.0–25.0	4.9	69.9	–0.66**
Thousand-kernel weight	15.8	0.0–35.8	5.6	48.9	–0.43
Average seed no. on side tillers	4.3	0.0–18.7	10.0	35.5	–0.73**
Grain yield (g)	0.7	0.0–4.8	1.7	28.0	–0.60*

(1) Value of significant difference between individual accessions at the P=0.05 level; (2) % of the vernalized treatment; (3) Correlations between the difference in unvernalized and vernalized treatments for a given trait and the level of vernalization responses (heading date 18 h uv – heading date 18 h vern); +, *, **: significant at the P=0.10, P=0.05 and P=0.01 levels, respectively.

The effect of various photoperiod regimes on agronomic traits was measured in vernalized treatments only. All the developmental and agronomic traits were significantly influenced by changes in the photoperiod regime. A comparison of the two group averages demonstrated that the *spontaneums* and barley cultivars significantly differed from each other in their reaction to shortening photoperiods (Fig. 1). In the cultivars the plant height and the last internode length showed a continuous decrease as the daylength decreased (Fig. 1a). Their node number increased significantly up till the 10 h photoperiod regime. The plant height, last internode length and node number of the *spontaneums* gave relatively constant values in the 12–18 h photoperiod ranges; with shorter daylength, however, the first two traits declined significantly, while the node number increased sharply, reaching the same level as in the cultivars.

In the case of the tillering ability at first node appearance (TIL31) and heading (TIL49) the two groups responded similarly when the photoperiod was between 12 and 18 hours (Fig. 1b). Under short photoperiod regimes, however, the *spontaneums* exhibited a very strong increase in TIL31, which reached over 400% of the initial value with the 8-hour photoperiod. In the case of TIL49, the highest value (over 250%) was recorded for the 10-hour photoperiod, dropping back to 200% in the 8-hour regime. RT remained relatively constant through all the photoperiod regimes. In the case of cultivars, TIL31 and TIL49 showed a significant increase as the photoperiod decreased, followed by a significant increase in RT till a photoperiod regime of 12 h. Under shorter photoperiods (10 and 8 h) a large ratio of the tillers died during the plant life cycle. Thus, TIL49 remained at a constant level, but RT (tillers bearing ears) significantly decreased.

All the yield components had significantly higher values in the cultivars than in the *spontaneums* under long photoperiods (Fig. 1c). At the same time, the cultivars showed a much greater sensitivity to changes in the photoperiod, all their yield components decreasing dramatically with decreasing daylength. The seed number in the main and side tillers was the most sensitive. The TKW showed a relatively small decline as the photoperiod shortened from 18 to 10 hours, but dropped to zero under the 8 h photoperiod. In the case of *spontaneums*, the yield components did not vary greatly in the different photoperiod regimes, the only exception being TKW, which declined to a larger extent.

When the reactions of the individual accessions and cultivars were considered, two *spontaneums*, Hvs3 and Hvs5, showed a significantly different pattern from the averages of all the *spontaneums* (Fig. 2). The pattern for the main tiller seed number of Hvs3 was more similar to the reaction type of the cultivars, while the TKW of Hvs5 was not significantly different from the average value of the cultivars, and its grain yield also showed a pattern very similar to that of the cultivars.

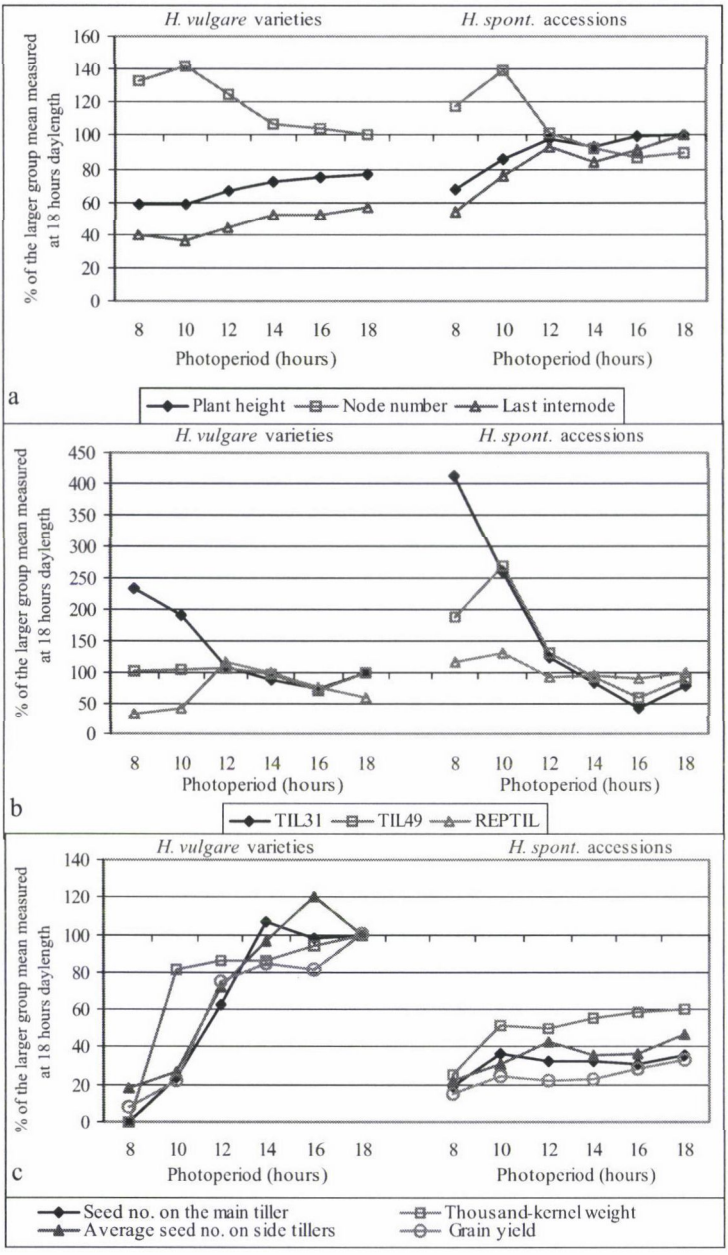

Fig. 1. Changes in plant developmental and yield component traits in groups of 16 *H. spontaneum* accessions and 8 *H. vulgare* varieties as a function of photoperiod regime (the larger group mean value of a given trait measured in the 18 h vernalized treatments represents 100%); (a) plant height and its components, (b) number of tillers at different plant developmental stages, (c) yield component traits

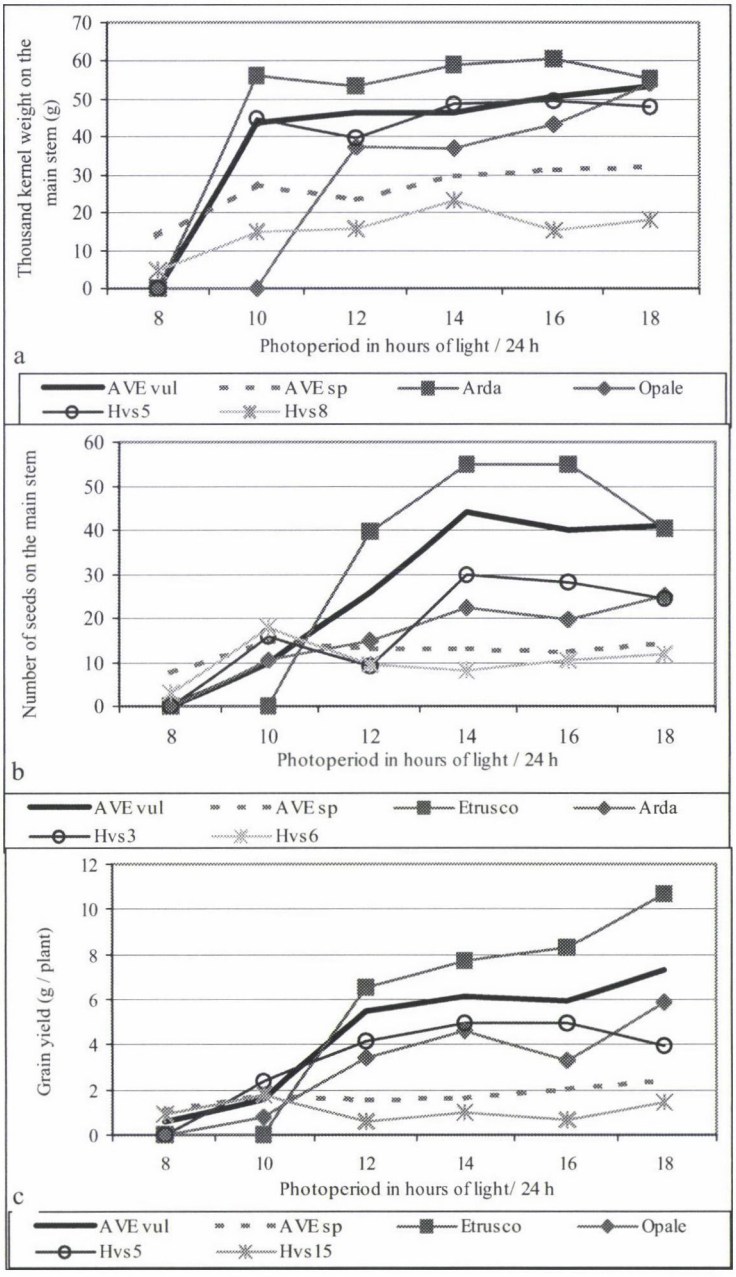

Fig. 2. Changes in average, minimum and maximum values of yield component traits in groups of 16 *Hordeum spontaneum* accessions (AVE sp) and 8 *Hordeum vulgare* cultivars (AVE vul) under different photoperiod regimes. (a) Thousand-kernel weight; (b) Seed number in the main stem; (c) Grain yield/plant

Discussion

In general, grain yield is increased by factors that allow longer periods for the production of more spikelets and fertile side tillers and/or allow longer grain filling periods (Snape et al., 1985; Worland, 1996). In wheat the effect of vernalization response was only found to be significant under favourable environmental conditions (Kato et al., 2000), when the later flowering allele was associated with an increased number of tillers and spikelets; but in several experiments its effect on grain yield could not be proved, probably due to the fact that the increase in spikelet number was counteracted by a reduction in grain size (Snape et al., 1985; Kato et al., 2000). In a range of *H. spontaneum* accessions vernalization had an overwhelming effect on heading date (Karsai et al., 2004), being of key importance for the transition from vegetative to reproductive growth. In addition to flowering, a whole range of plant developmental and agronomic traits were found to be determined by vernalization, with severely impaired reproductive fitness when the vernalization requirements of the plants were not saturated. Variation in the magnitude of vernalization response was found between accessions from the Fertile Crescent, but much of this variation was observed at photoperiod durations uncharacteristic of the region (Karsai et al., 2004). However, this variation showed a significant correlation with the sensitivity of several yield component traits to the lack of vernalization. A larger decrease in the reproductive tiller number, average seed number and consequently the final grain yield was much more characteristic of accessions with greater vernalization responses.

In addition to vernalization, photoperiod also has a significant effect on reproductive fitness and agronomic traits. Detailed studies on the effect of the *Ppd-1* photoperiod response locus produced contrasting results. Worland (1996) demonstrated a complementary effect of the *Ppd-D1* locus in wheat, as insensitive lines with a shortened life cycle showed a significant increase in spikelet fertility, resulting in higher seed number in spite of the reduced number of spikelets. In barley the effect of the insensitivity allele at *Ppd-H1* was different, as it resulted in later flowering (Laurie et al., 1994; Karsai et al., 1999). Laurie et al. (1994) found that this late heading was accompanied by increased plant height, plant grain weight, tiller grain weight and ear grain number, showing the positive agronomic value of a longer growing period on the yield components of insensitive lines. In another barley population photoperiod insensitivity resulted in a larger grain number per ear, but this was accompanied by a smaller thousand-kernel weight, so the final effect of the *Ppd-H1* locus on plant yield proved to be insignificant (Karsai et al., 1999). In studying the determinants of heading date in *spontaneums* it was found that photoperiod sensitivity was expressed only when the vernalization requirements were fulfilled (Karsai et al., 2004). This underscores the fact that photoperiod sensitivity complements, but does not substitute for, vernalization. This mode of action could be detected in the case of plant developmental and agronomic traits. Under optimal growing conditions (e.g. optimal temperature, nutrient and water

supply, fulfilled vernalization requirement) a long photoperiod enhanced the reproductive fitness of the plants and resulted in larger yields and yield components, irrespective of the genotype, while short photoperiods represented limiting factors for all these traits. The *spontaneum* accessions and barley cultivars exhibited different responses to the shortening of the photoperiod, reflecting the different strategies characteristic of wild and domesticated species (Kato et al., 1997). For wild species the maintenance of reproductive fitness is the major goal, so genotypes able to maintain similar reproduction rates under diverse conditions have favourable selection potential (Karsai et al., 2004). Among the *spontaneums* tested, the number of reproductive tillers and the average seed number per tiller were kept relatively constant at the cost of a decrease in thousand kernel weight. Domestication, selection and breeding have led to different priorities, higher yielding ability becoming the major goal. The adaptability of cultivated varieties has also decreased significantly parallel to the more specific environmental requirements for the manifestation of their yielding ability. Thus, in the present experiment the cultivars were more sensitive to a shortening of the photoperiod than the wild genotypes. In addition, among the yield components, the thousand-kernel weight remained relatively constant in the cultivars at the cost of a decrease in tiller number and average seed number.

Two *spontaneum* accessions showed unique patterns of reproductive fitness traits. Hvs3 from Tibet, which has spring growth habit, produced a high seed number on the main tiller similar to that of the two-rowed cultivars, which may be the result of its origin. Von Bothmer et al. (2003) indicated that the eastern margin of *spontaneum* distribution was Afghanistan and Pakistan, but not Nepal or China. It is probable that the Himalayan *spontaneum* grew as a weed in its original distribution area and was harvested and mixed with cultivated barley seed, thus being introduced into the Himalayan region. In this environment, *spontaneum* may have persisted as a weed in the cultivated forms, which could have induced cross-pollination and the fixation of some domesticated traits. The other *spontaneum* accession, Hvs5, showed a yielding ability comparable to cultivars in these controlled environment tests. Co-existence with cultivated forms could also be the case for Hvs5, the exact origin of which within Israel is not known.

In conclusion a combination of photoperiod sensitivity and vernalization response was proved to have a significant effect on plant developmental and agronomic traits in this group of *spontaneum* accessions and barley cultivars. There was, however, a marked difference in the reaction type of wild and cultivated genotypes due to different plant strategies.

Acknowledgements

The seeds of the *spontaneum* accessions, Harrington, Strider, Kold and 88Ab536, were kindly provided by Professor Patrick M. Hayes, for whose help and advice we are grateful, while the seeds of Arda, Opale, Etrusco and Pirate were obtained from Dr Roberto Tuberosa.

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Acta Agronomica Hungarica, 53(1), pp. 99–107 (2005)

EFFECT OF CYANOBACTERIAL AND MICROALGAL BIOMASS ON ANTHER CULTURE RESPONSE OF WHEAT (*TRITICUM AESTIVUM* L.)

K. JÄGER[1], V. ÖRDÖG[2] and B. BARNABÁS[1]

[1]AGRICULTURAL RESEARCH INSTITUTE OF THE HUNGARIAN ACADEMY OF SCIENCES, MARTONVÁSÁR, HUNGARY;
[2]FACULTY OF AGRICULTURAL AND FOOD SCIENCES, UNIVERSITY OF WEST HUNGARY, MOSONMAGYARÓVÁR, HUNGARY

Received: 18 March, 2005; accepted: 2 May, 2005

In the present work the effect of microalgal and cyanobacterial biomass on anther cultures of wheat (*Triticum aestivum* L.) was studied. Investigations were made on the influence of media supplemented with a biomass of four terrestrial and fresh-water microalgal and cyanobacterial strains (1–2 g L^{-1}) on the androgenic response, frequency of microspore-derived embryo-like structures, and their regeneration capacity in anther cultures of wheat. The addition of 1 g L^{-1} of cyanobacterium biomass MACC 643 to the induction and regeneration media significantly improved the androgenic response by 50% in the genotype Mv Pálma, and reduced the requirement for the synthetic auxin 2,4-D.

Key words: anther culture, cyanobacteria, microalgae, *Triticum aestivum* L., wheat

Introduction

Plant physiologists, embryologists, geneticists and breeders have shown great interest in androgenesis (the process by which plants are produced from microspores) and haploids since the first discovery of natural haploid plants in *Datura stramonium* as early as 1922 (Blakeslee et al., 1922). Similarly, after the first report describing embryogenesis from the pollen of *Datura innoxia* by Guha and Maheshwari (1964), isolated anther culture has become an important tool for the production of varieties with doubled haploidy in several important crops (Thomas et al., 2003). The plant growth regulator 2,4-D is widely used in anther culture induction media due to its reliably positive effect on androgenesis. However, 2,4-D causes a rapid dose-dependent decrease in the number of dividing cells, cell membrane damage, cytoplasm and nucleoplasm vacuolisation, as well as chromatin abnormalities and chromosome aberrations (Ateeq et al., 2001). Chlorophyll deficiency and pollen sterility were also observed in *Allium cepa* and *Oryza sativa* regenerants after treatment with higher doses of 2,4-D (Kumari and Vaidyanath, 1989). High auxin concentrations increased the anther culture response in maize, but exogenous growth regulators were reported to decrease the frequency of maize embryo-like structures (Büter, 1997). Differences in the embryogenic competence of genotypes may be caused by the differential response of the cells to exogenous plant growth regulators, especially to 2,4-D (Dolgykh, 1994). The hormone

0238–0161/$ 20.00

requirement may also be genotype-dependent (Rakoczy-Trojanowska et al., 1997) and the growing conditions of donor plants may influence these requirements (Ferrie et al., 1995). One of the major differences between the more and less competent lines is the distribution of 2,4-D within the embryos after culturing them on medium containing 2,4-D (Bronsema et al., 1998). In carrot cells, exogenous 2,4-D stimulated the accumulation of large amounts of endogenous IAA (Michalczuk et al., 1992). These authors hypothesized that the embryogenic competence of carrot cells was closely associated with a several-fold increase in endogenous IAA levels due to the presence of 2,4-D. A higher endogenous indole-3-acetic acid (IAA) level has also been shown to be associated with increased embryogenic response in maize (Jiménez and Bangerth, 2001). It was suggested that this synthetic compound acted indirectly by interfering with the endogenous auxin metabolism, while the direct auxin effect of 2,4-D was less significant. In alfalfa leaf protoplasts cultured in the presence of 2,4-D, the endogenous IAA levels increased considerably during the first 2–3 days of culture (Pasternak et al., 2002). The immuno-cytochemical localization of IAA in the immature zygotic embryos provided direct evidence that an endogenous auxin pulse may be one of the first signals leading to somatic embryogenesis (Thomas et al., 2002). The polar transport of auxin is essential for the establishment of bilateral symmetry during embryogenesis in dicotyledonous and monocotyledonous species (Fischer and Neuhaus, 1996). Embryogenesis might be triggered by the exclusion of the 2,4-D concentration in the culture medium of embryogenic cultures, to allow the establishment of the polar auxin gradient (Jiménez, 2001). Filippini et al. (1992) reported a reduction in the embryogenic capacity of the explants with time in culture on medium containing 2,4-D.

Natural extracts from higher plants are widely used as additives in cereal anther cultures. Potato-2 medium (Chuang et al., 1978), which contains a 10 % potato extract, has been routinely used in anther culture induction media for various cereals. Barley starch, when added to barley induction and regeneration media, increased the number of responding anthers and reduced the frequency of albino regenerants (Sorvary, 1986). Similar results were obtained when using wheat, rice and potato starch (Sorvary, 1986).

Plant growth regulators (PGRs) occur in a wide range of organisms. PGRs from all five groups of growth substances have been identified in numerous macroalgae (Bradley, 1991) and there is no reason why unicellular species should not both synthesize and use growth substances during their limited development (Evans and Trewavas, 1991). Stirk et al. (2002) detected both cytokinin- and auxin-like activities of terrestrial and fresh water cyanobacteria and microalgae using bioassays. The cytokinin content of nine microalgal strains from the genera *Protococcus, Chlorella* and *Scenedesmus* was confirmed by Ördög et al. (2004). The production of phytohormones is also widespread among soil- and plant-associated prokaryotes (Costacurta and Vanderleyden, 1995).

Some free-living and, in particular, symbiotic cyanobacteria are able to accumulate and release the phytohormone indole-3-acetic acid (Sergeeva et al., 2002). So far no microalgal or cyanobacterial biomass has been used in anther cultures of cereals.

The aim of the present study was to examine the potential of cyanobacterial and microalgal biomass to enhance the efficiency of the anther response and the frequency of microspore-derived embryo-like structures and regenerants.

Materials and methods

Plant material

Anthers from field-grown donor plants of the winter wheat cultivar Mv Pálma produced in the Agricultural Research Institute, Martonvásár, and the cultivar Benoist (H77022) were used in the experiment. According to the data obtained from the meteorological station located in the research institute, during the first decade of May 2003 the temperature was 5.1°C higher than the thirty-year average. In addition, there were four hot days (with temperatures exceeding 30°C) prior to collecting the wheat spikes. A precipitation sum of 14.8 mm was recorded during April and the first ten days of May, which was only 32% of the average rainfall.

In vitro *culture*

Wheat spikes were collected and surface sterilized with 2% (v/v) sodium hypochlorite for 20 min and washed three times with sterile distilled water. The late uninucleate stage of microspore development was determined under a light microscope after staining with acetocarmine. Anthers were dissected under sterile conditions and inoculated onto a W_{14} (Ouyang et al., 1989) induction medium supplemented with 1 g L^{-1} biomass of the microalgal strains MACC 553 (*Klebsormidium* sp.) and MACC 583 (*Neochloris* sp.) and the cyanobacterial strains MACC 642 (*Leptolyngbya* sp.) and MACC 643 (*Anabaena* sp.) as a plant growth substance, combined with 1 mg L^{-1} 2,4-D and 0.25 mg L^{-1} kinetin. In a second treatment, 2 g L^{-1} biomass was added without using synthetic hormones. W_{14} medium containing 2 mg L^{-1} 2,4-D and 0.5 mg L^{-1} kinetin was used as the control.

Isolated anthers were incubated at 29°C for 4 weeks in the dark. In each treatment 150 anthers were plated per Petri dish in four replicates. After the four-week incubation the frequency of anther induction and microspore-derived embryo-like structures was calculated as a percentage of the plated anthers.

After the induction period the microspore-derived structures were transferred into 190-2 regeneration medium (He and Ouyang, 1983). The original synthetic hormone content (0.5 mg L^{-1} kinetin plus 0.5 mg L^{-1} 1-naphthylacetic acid) of the regeneration medium was halved or replaced by 1 or 2 g L^{-1} cyanobacterial and microalgal biomass. After the four-week incubation period the frequency of regenerants was calculated as a percentage of the plated anthers. All data were pooled as means from the four replicates, and statistically analysed with ANOVA (SPSS for Windows, version 10.0).

Cultivation of microalgal and cyanobacterial strains

Batch cultures of the microalgal MACC (Mosonmagyaróvár Algal Culture Collection) strains 553 (*Klebsormidium* sp.) and 583 (*Neochloris* sp.), and the cyanobacterial strains 642 (*Leptolyngbya* sp.) and 643 (*Anabaena* sp.), selected previously as having a biomass production of >1.5 g L^{-1} during culture, were grown in an apparatus for laboratory algal bioassay described earlier (Ördög, 1981). The cultures were incubated at 25 ± 2°C, with a 12:12 h light-and-dark cycle, illuminated from below with a light intensity of 130 µmol $m^{-2}s^{-1}$. The cultures were aerated

with 25 L compressed air per hour through sterile cotton filters and mixed twice a day. The algal biomass was harvested in the early stationary phase of growth by centrifugation for 15 min at 3,500 rpm at the same time of a day. The supernatant-free biomasses were freeze-dried for 22 h at 0.035 mbar and stored at –20°C. The freeze-dried samples were re-suspended in distilled water, ultra-sonicated for 2 min and diluted to 2 g L^{-1} dry matter concentration.

Bioassays of microalgal and cyanobacterial strains

The excised cucumber cotyledon bioassay (Zhao et al., 1992) was used to determine the cytokinin- and auxin-like activities of the MACC strains. Cucumber seeds were germinated in the dark on Knop medium (Knop, 1865) solidified with 6 g L^{-1} agar. Cotyledons were excised from 5-day-old seedlings. Ten cotyledons were placed under green light in a 6-cm Petri dish on the surface of a filter paper disc. The cotyledons were treated with 3 ml solution of 6-furfuryl-amino-purin (kinetin) or indole-3-butyric acid (IBA) at concentrations of 0.1, 0.3, 0.5, 1, 3, 5 and 10 mg L^{-1}, and with microalgal or cyanobacterial suspensions (2 g L^{-1}). Distilled water was used as the control. The cotyledons were incubated in the dark (25 ± 2°C) for 3 and 5 days before the determination of the cytokinin- and auxin-like activity, respectively. The fresh weight of 10 cotyledons was recorded and compared to the activity of a kinetin standard series. The number of roots was counted and compared to various IBA concentrations. The experiment was repeated independently three times with four replications in each treatment. All data were pooled as means from the three replicates, and statistically analysed with ANOVA (SPSS for Windows, version 10.0).

Results and discussion

Auxin- and cytokinin-like activity of microalgal and cyanobacterial strains

All the microalgal and cyanobacterial strains used in the experiment showed both auxin- and cytokinin-like activities compared with the control (Figs. 1 and 2). The MACC 643 cyanobacterial strain showed the highest (128%) cytokinin-like activity (Fig. 1).

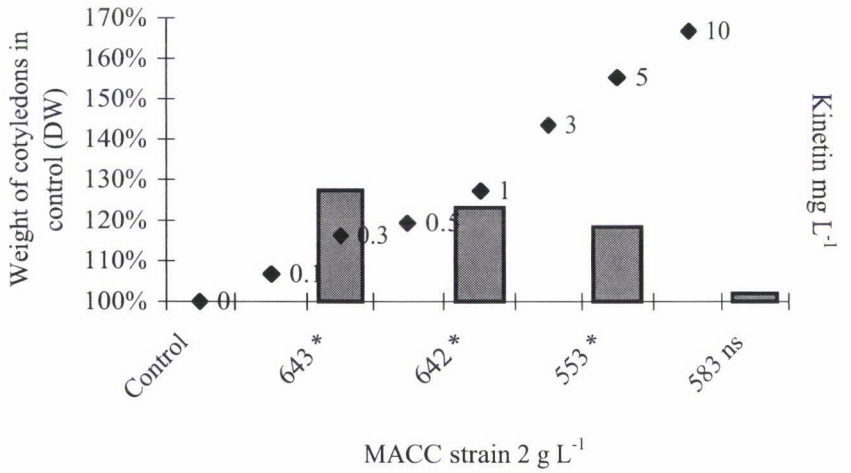

Fig. 1. Cytokinin-like activities of the MACC strains investigated based on the expansion of excised cucumber cotyledons, compared to that induced by different kinetin concentrations.
* significant at the P<0.05 level of probability; ns not significant; control: distilled water

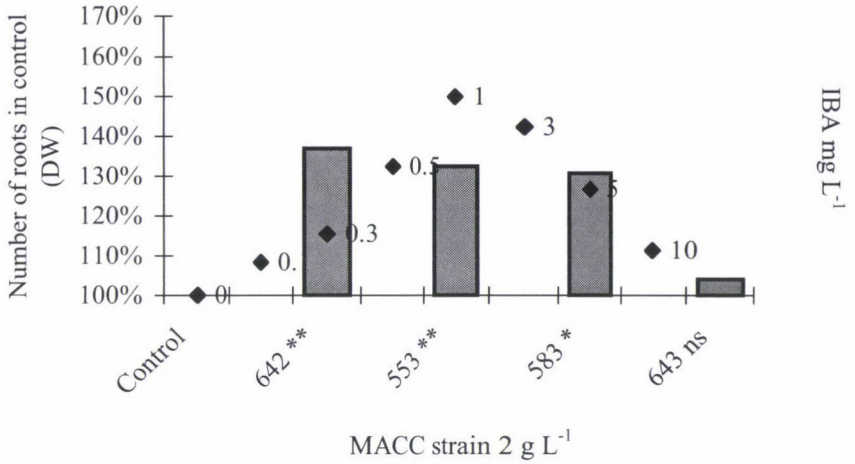

Fig. 2. Auxin-like activities of the investigated MACC strains based on the root formation of excised cucumber. **,* significant at the P<0.005 and P<0.05 levels of probability; ns: not significant; control: distilled water

The MACC strains 643, 642 and 553 significantly increased the expansion of cucumber cotyledons at the P<0.05 level of probability compared to the distilled water used as the control. The kinetin equivalent of different cyanobacterial and microalgal strains could be determined by comparing the increase in cucumber cotyledon weight caused by MACC strains and a kinetin standard series.

The auxin-like activities of the cyanobacterial strains MACC 642 and 643 were significantly higher at the P<0.005 level of probability compared to the effect of the control. Treatment with MACC 583 biomass gave a 31% increase in auxin-like activity at the P<0.05 level of probability in the excised cucumber cotyledon root formation bioassay. The indole-3-butyric acid (IBA) equivalent of different strains could be determined by comparing the frequency in root initiation caused by MACC strains and by different IBA concentrations.

Effect of microalgal and cyanobacterial biomass on anther culturability of wheat

All the parameters indicative of the anther culture response (anther response, embryo-like structures and regenerants) of the genotypes used in the experiment were lower than expected (Table 1). The low frequency of anther induction, and especially that of embryo-like structures and regenerants, was due to the severe climatic conditions, in accordance with data previously reported by Nitsch et al. (1982) and Genovesi (1990).

Under control conditions, an average of 20 anthers responded, 34 microspore-derived embryo-like structures emerged and 4.4 plants were regenerated per 100 plated anthers in the genotype Benoist (Table 1). Treatments containing 1 g L^{-1} MACC 642 biomass + 1 mg L^{-1} 2,4-D, 1 g L^{-1} MACC 643

biomass + 1 mg L^{-1} 2,4-D and 2 g L^{-1} MACC 643 surpassed the anther induction frequency of the control, but the increase was not significant. When other cyanobacterial and microalgal biomasses were added to the induction medium the anther induction did not reach the control value, but the decrease (except for treatment with 2 g L^{-1} MACC 583 biomass, which caused a 75% decrease) was not significant. Treatments containing 1 g L^{-1} MACC 642 biomass + 1 mg L^{-1} 2,4-D, 1 g L^{-1} MACC 643 biomass + 1 mg L^{-1} 2,4-D and 2 g L^{-1} MACC 643 biomass also enhanced the frequency of embryo-like structures by 17%, 10% and 17%, respectively, which were not significant. When adding 2 g L^{-1} MACC 583 biomass to the induction medium, a significant 68% decrease in the number of embryo-like structures was detected. When 1 g L^{-1} MACC 642 or 643 biomass + 1 mg L^{-1} 2,4-D were added to the regeneration medium, the number of regenerants increased by 12% and 17 %, respectively (Table 1).

The same treatments resulted in an increase in anther induction in the cultivar Mv Pálma (Table 1) as in the case of Benoist. The positive effect of treatment with 1 g L^{-1} MACC 643 biomass + 1 mg L^{-1} 2,4-D on anther induction was significant at the $P<0.05$ level of probability. This treatment also surpassed the control in the frequency of embryo-like structures and regenerants, but the differences were not significant. Similarly to the effect found in Benoist, MACC 583 biomass caused a 50% and 52% decrease in the frequency of anther induction and embryo-like structures, respectively, when added to the induction medium at a concentration of 2 g L^{-1}.

Table 1

Effect of anther induction and regeneration media supplemented with microalgal and cyanobacterial biomass on the frequency of anther induction, embryo-like structures and regenerants in wheat cultivars Benoist and Mv Pálma grown under natural climatic conditions, as a percentage of the control

Treatment	Wheat cultivar					
	Benoist			Mv Pálma		
	Responding anthers	Embryo-like structures	Regenerants	Responding anthers	Embryo-like structures	Regenerants
	as a % of plated anthers					
Control	20	34	4.4	10	23	1.5
1	15ns	38ns	nt	9ns	24ns	nt
2	12ns	28ns	1.0ns	6ns	19ns	0.3ns
3	16ns	29ns	0.6ns	9ns	23ns	nt
4	5*	11*	nt	5*	11*	nt
5	25ns	41ns	5.0ns	10ns	18ns	1.5ns
6	12ns	24ns	0.9ns	7ns	16ns	nt
7	27ns	39ns	4.4ns	15*	26ns	1.8ns
8	22ns	41ns	0.9ns	11ns	22ns	nt

Control: 2 mg L^{-1} 2,4-D; Treatments: 1: 1 g L^{-1} MACC 553 + 1 mg L^{-1}2,4-D, 2: 2 g g L^{-1} MACC·553; 3: 1 g L^{-1} MACC 583 + 1 mg L^{-1} 2,4-D; 4: 2 g L^{-1} MACC 583; 5: 1 g L^{-1} MACC 642 + 1 mg L^{-1}2,4-D; 6: 2 g L^{-1} MACC 642; 7: 1 g L^{-1} MACC 643 + 1 mg L^{-1}2,4-D; 8: 2 g L^{-1} MACC 643; *Significantly different from the control at $P<0.05$; ns not significant; nt not tested

Differences were observed between the combinations of the given microalgal or cyanobacterial biomasses. The treatments containing 1 g L^{-1} MACC biomass + 1 mg L^{-1} 2,4-D slightly increased the number of responding anthers and embryo-like structures compared to treatments without 2,4-D in both genotypes (Table 1).

When comparing the results of the cucumber cotyledon expansion bioassay (Fig. 1) with the anther culture experiments (Table 1), a correlation can be found between the cytokinin-like activities of the MACC strains and their impact on the anther culturability of the two wheat cultivars. The MACC 643 strain, which showed the highest cytokinin-like activity, gave the highest anther induction and regeneration frequency. In contrast, biomass of the MACC 583 strain showed almost no cytokinin-like activity, and when it was added to the anther induction and regeneration media, all the parameters of anther response were lower at the $P<0.05$ level of probability in both genotypes compared to the control.

Biomass of the microalgal (MACC 553 *Klebsormidium* sp.) and cyanobacterial (MACC 642 *Leptolyngbya* sp., MACC 643 *Anabaena* sp.) strains used as additives in this experiment were able to reduce the synthetic auxin 2,4-D requirement. As anther culture represents a modern tool for the rapid achievement of homozygosity and for obtaining doubled haploids for the improvement of cultivated species, the optimisation of the wheat anther culture protocol using microalgal and cyanobacterial additives will be of considerable interest to plant breeders. Quantitative and qualitative analysis of the bioactive compounds produced by the microalgae and cyanobacteria used in the experiments will be required to determine the factors causing an increase in the wheat anther culture response. Verification of the chemical authenticity of these active compounds will allow their application to enhance the efficiency of anther and microspore cultures.

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Acta Agronomica Hungarica, 53(1), pp. 109–118 (2005)

Review

EFFECT OF POPCORN VARIETIES FROM THE ANDES ON THE DEVELOPMENT OF THE EARLY, HARD-GRAINED GENE POOL IN CENTRAL EUROPE

G. HADI

AGRICULTURAL RESEARCH INSTITUTE OF THE HUNGARIAN ACADEMY OF SCIENCES, MARTONVÁSÁR, HUNGARY

Received: 19 November, 2004; accepted: 28 April, 2005

The ancestors of the early, multi-rowed, hard-grained flint maize varieties found in Central Europe, and also of some of the dent varieties, were in all probability Chutucuno Chico and Chutucuno Grande (Timothy et al., 1961), chilling-tolerant, daylength-insensitive, small-eared, multi-rowed, prolific, hard flint popcorn varieties with reddish-brown kernels originating from the slopes of the Andes and introduced into Hungary in the early 1800s via Italy (Nagyváthy, 1822). In Italy and Hungary these varieties were given the names Cinquantino and Pignoletto.

In addition to these Andean sources, a considerable contribution to the hard-grained gene pool was also made by Hungarian flints of Caribbean origin, and to a lesser extent by Southern Dents and Corn Belt Dents, while Northern Flints played little role in its development. These maize varieties were grown chiefly for human consumption (in the form of porridge) and were exported to Italy, Slovenia and Romania. The high price paid for exported maize, the low yield and undesirably long vegetation period of the initial sources, and the need to improve the colour and ear fusarium resistance of commercial maize meant that breeding was begun as early as the 1850s. The data available indicate that in around 1856, probably for the first time in Europe but independently of each other, Pál Németh and Pál Máthé crossed Cinquantino with other varieties in order to produce new varieties. These varieties, and those later selected from them, played a role in the maize production of Hungary, Romania, Yugoslavia, Slovakia and other countries for a period of 100 years. In addition, they probably exerted a significant effect on the early flint gene pools of all European countries where the vegetation period is either extremely short or extremely cool.

Key words: maize, breeding, source of heterosis

Introduction

Knowledge of the pedigree or breeding history of maize varieties may assist breeders to utilise the breeding stock more efficiently (Troyer, 1999; 2004). Due to the lack of investigations on this subject, many breeders assume that the gene pool of early European flints originates from the Northern Flints. However, if the morphological appearance of the three gene pools used most widely in the development of early silage maize hybrids (Ep 1 from Spain, F2 and F7 from France, DK 105 from Germany) is compared with that of the Northern Flints (Brown, 1947), numerous fundamental differences can be observed. Little information is available on the development of the early European flints (Jánossy et al., 1957; Pavlicic, 1971; Brandolini, 1971; Brandolini and Avila, 1971; Trifunovic, 1978; Hadi et al., 2003; Hadi, 2004; Marton and Szél, 2003).

0238–0161/$ 20.00

Breeding experience suggests that chilling-tolerant, early flowering soft flints with 14–16 kernel rows, pointed ears and short erect leaves, originating from hilly regions of Mexico or Guatemala, may have been involved in the development of the Ep 1 gene pool. The similarity in the morphological appearance of hybrids developed using the French lines F2 and F7, and perhaps the German line DK 105, and those produced from certain hard-grained, early, multi-rowed Central European flints supports the hypothesis that the former may have developed parallel or jointly with the latter. The early, multi-rowed Central European flints may have participated in the creation of the early European flints, which differ from the Northern Flints. The confirmation or refutation of this theory using other methods could be useful for the further improvement of the early European flint gene pool.

Materials and methods

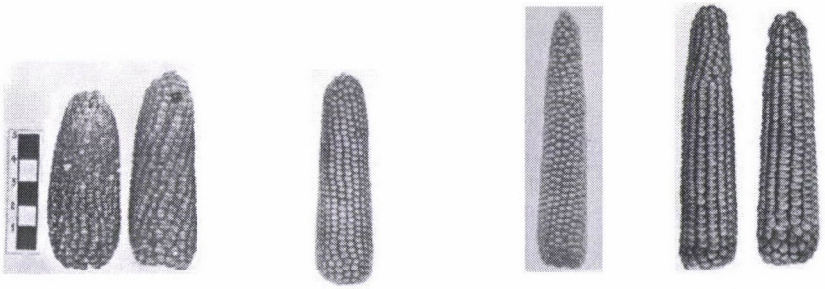
One important source of data was published by Ábrány et al. (1955), who collected and catalogued Hungarian works on maize from the very earliest times. Basic data and photographs illustrating the origin and development of the varieties were taken from the publications of numerous maize breeders and growers. The importance of these varieties in maize production was clear from works by Szentkirályi (1881; 1891; 1894; 1895; 1896; 1899; 1901) and Balogh (1988). When various reports were contradictory, further literary sources were checked, and data were accepted as valid if they fitted logically into the flow of events.

Results

The Cinquantino group of varieties

Varieties based on Cinquantino (Figs. 1–4) were grown chiefly on small farms for human consumption and export purposes. These varieties flowered earliest in Hungary and had somewhat softer kernels than the Pignoletto varieties.

In around 1856 a Hungarian farmer from Hódmezővásárhely, Pál Németh, began to make crosses to develop new basic stock. He planted alternate rows of a locally developed variant of Old Hungarian Yellow Flint, a late-maturing, large-eared variety of the Caribbean type with 8–12 kernel rows, and the early-maturing, prolific variety Cinquantino, acquired from Derekegyháza (Hungary), which had small, reddish kernels and ears measuring only 4–5 cm. The difference in the flowering time was overcome by sowing at different dates. This cross was repeated on several occasions. Early-maturing plants with large ears and hard pink kernels were selected from the hybrids, and by 1867 a variety with 2–3 ears, each 16–20 cm in length, with 12–16 rows of semi-hard, brightly coloured kernels had been developed, which was widely grown on farms in the region.



Chutucuno Chico (Source: Thimothy et al., 1961) | Cinquantino (Source: Villax Ö.-Surányi J., 1932) | Legkorábbi székely (Source: Grabner E., 1908) | Putyi Basa (Source: Villax Ö.-Surányi J., 1932)

Fig. 1. Varieties related to Cinquantino

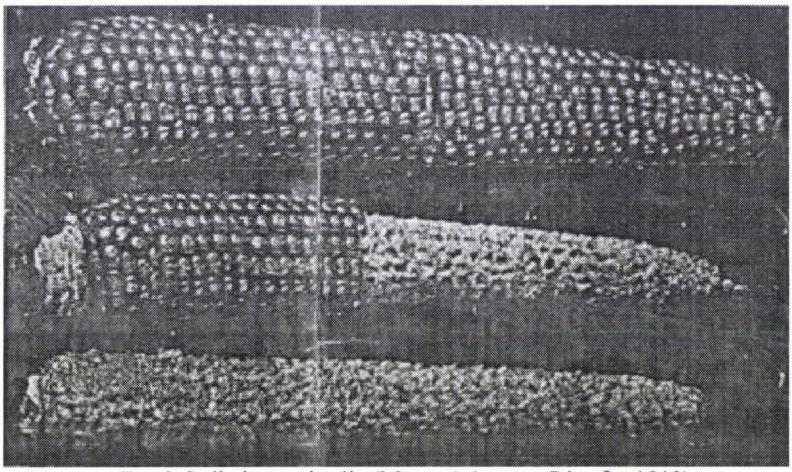


Fig. 2. Székely muskotály (Muscat) (source: Péterfy, 1913)

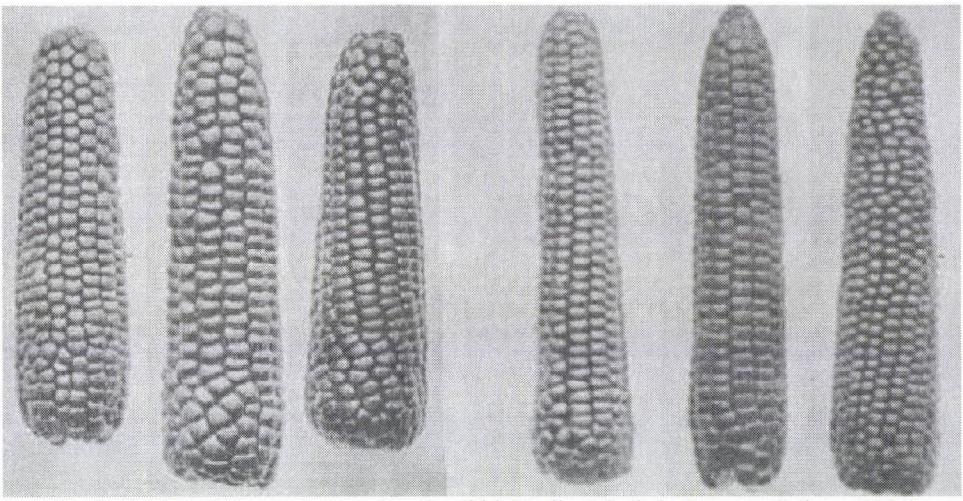


Fig. 3. Thick and long ear types of Legkorábbi (Earliest) Székely (source: Grábner, 1908)

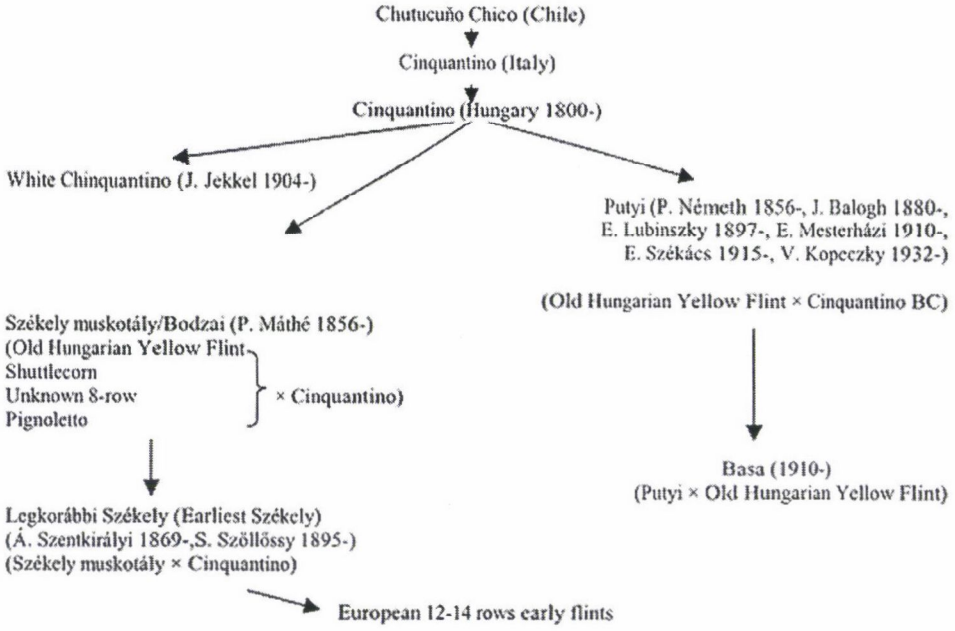


Fig. 4. Origin of the Cinquantino group

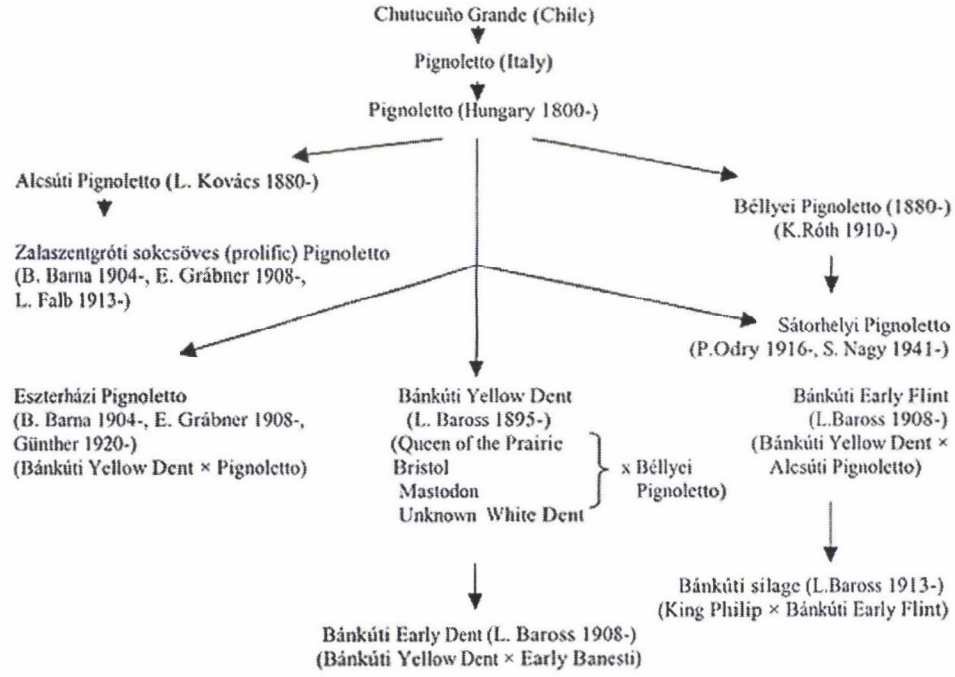


Fig. 5. Origin of the Pignoletto group

The vegetation period of this new variety was, however, still longer than desirable. A farmer called Balogh (nicknamed Putyi) carried out selection for early-flowering, early-maturing plants. From 1880 onwards he gradually developed a large-eared, hard-grained maize variety with extra early maturity, known in the Hódmezővásárhely area as Putyi or, because of its colour, Rózsa (Rose) (Péterfy, 1912b). This variety soon spread and retained its popularity for a long period. It was further improved by Ernő Lubinszky (from 1897–1910), Ernő Mesterházi, (from 1908–1924), Elemér Székács (from 1915–1932) and Viktor Kopeczky (after 1938). According to the data published by Balogh (1988) this variety was grown on 10% of the maize sowing area in 1938–1945 and on 2.5% in 1946–1962. Putyi was the most popular early variety in Hungary for nearly 100 years, from 1867–1962. Due to its valuable traits it was also used as a crossing partner in the development of new varieties. The dent variety Pettendi Aranyözön, which occupied 10% of the sowing area for 25 years, probably inherited its earliness, chilling tolerance and drought tolerance from Putyi. Through natural mixing on farms with local variants of Old Hungarian Yellow Flint, it also made a substantial contribution to the development of the prolific, drought-tolerant variety Basa, which had hard flint kernels.

The other extra-early maturing variety developed from Cinquantino was Legkorábbi (Earliest) Székely, which became surprisingly well known internationally. The breeder of this variety, Árpád Szentkirályi, who owned an estate in Székelyudvarhely (Transylvania), found a relatively early flowering maize variety in a valley in the Bodza Mountains (short season, cold location), which appeared to be a good source for earliness, kernel colour and kernel type. This was achieved by crossing it with Cinquantino, which flowered somewhat later, but had bright pink, hard kernels (Szentkirályi, 1881). According to Péterfy (1913), the variety found by Szentkirályi may have been identical with the maize called Bodzai by Pál Máthé, later also named Székely muskotály (Muscat).

Pál Máthé, who owned an estate in Bikafalva (Transylvania), started breeding maize by crossing at much the same time as Pál Németh, in 1856. He crossed four varieties (a local variant of Old Hungarian Yellow Flint, Shuttlecorn (probably a Northern Flint), an unidentified long-eared, 8-row variety (Northern Flint?) and Pignoletto with Cinquantino. By selecting early-maturing, large-eared plants with bright kernel colour, he developed a variety with 14–16-row ears 16–20 cm in length, remarkable both for its earliness and great yield potential (Péterfy, 1913). This variety was widely grown in the neighbourhood of Bikafalva even before it was put on the market. It was probably this variety that was crossed with Cinquantino in 1869 by Árpád Szentkirályi, who concentrated mostly on achieving early maturity, but also improved the prolificacy, yield potential and marketability of the variety using the mass selection method based on typical traits, which probably involved partial inbreeding (Szentkirályi, 1881). Besides being a successful breeder, Szentkirályi was also an excellent businessman (Szentkirályi, 1877a, b; 1881; 1891; 1894; 1895; 1896; 1899; 1901, etc.). He set up a Seed Production Company to multiply the seed of the variety Legkorábbi Székely and the seed

was marketed by such well-known seed merchants as Vilmorin & Andrieux and Skanska & Frökontoret in France (where the variety was known as M. Saint-Kirally), Ödön Mauthner and Frommer A. Hermann in Germany and the Austro-Hungarian Empire, and Clayton & Shuttleworth in Russia. The variety was given good publicity both at home and abroad (Gobóczy, 1879; 1880; Krocsák, 1881; Réti, 1888; Strebel, 1889; Fényes, 1889; Anonymus, 1896; Grábner, 1908). It was noted for its earliness and was ranked first for yield performance in numerous trials both in Hungary and abroad. It was awarded a Grand Prize at the Budapest Exhibition in 1885, First Prize and a Gold Medal at the Vienna Exhibition in 1890, Grand Prize at the Millenary Exhibition in Budapest in 1896, Grand Prize at the Brussels World Fair in 1897, Grand Prize at the Paris World Fair in 1900 and a Silver Medal at the First National Maize Exhibition in 1914. Unfortunately no data are now available on the criteria on which these awards were based, but it seems probable that the results achieved in competitive trials and its widespread popularity played an important role.

There was great demand from growers for seed of this variety, which was often multiplied illegally, as it could be sold for a high price. On occasion, as the supply of seed was always less than the demand, the seed was distributed to farmers by the government. In average years the Szentkirályi Company marketed over 100 tonnes of carefully selected certified seed. The variety was grown widely between 1890 and 1910 in regions of France, Germany, Austria, Bohemia, Poland and Russia where weather conditions made it necessary to grow especially chilling-tolerant, early-flowering, but high-yielding varieties.

The gene pool of Legkorábbi Székely was no doubt utilised throughout Europe in the development of both recognised and locally developed varieties. It is certainly not by chance that the ear morphology of the hybrids F2 and F7, derived from the variety Lacaune, and of DK 105, which originated from Badischer Landemais, bore a strong resemblance to that of Legkorábbi Székely (Figs. 2 and 3), the phenotype of which differs considerably from that of all the characteristic types of Northern Flints (Brown, 1947).

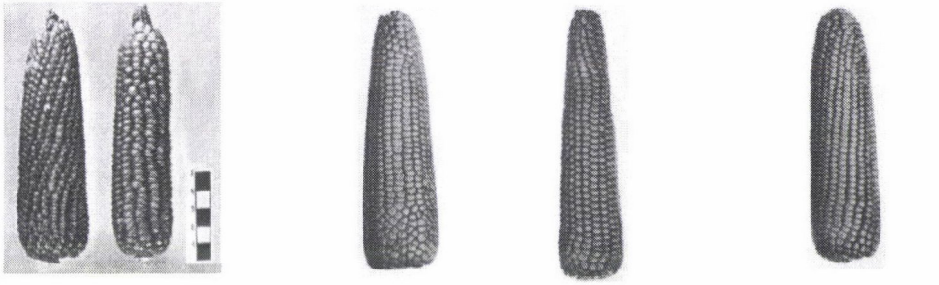
The Pignoletto group of varieties

The other major group of multi-rowed, hard-grained flints in Central Europe originated from Pignoletto (Figs. 5, 6 and 7) (Barna, 1904; Péterfy, 1912a, b; Grábner, 1913; 1916; 1917; 1922; 1929; Fabricius, 1921; Villax and Surányi, 1932). This chilling-tolerant, daylength-insensitive popcorn variety (used to make porridge), which had brown ears 12–14 cm in length with 16–18 kernel rows, had long been grown on the Béllye estate of Prince Joseph Habsburg (Croatia) before breeding was begun in around 1880. The name of the first breeder is unknown. In the 1910s the variety was bred by Károly Róth, and after the first world war, when the breeding stock was transferred to the prince's Hungarian estate in Sátorhely, the work was continued first by Pál Odry, who registered the variety under the name Sátorhelyi Pignoletto in 1929, and later, from 1941 onwards, by Sándor Nagy. Over a period of 20–30 years the originally late-maturing Pignoletto had become an early-maturing, high-yielding

variety with 2 or 3 conical ears each 16–18 cm in length, bearing 18–22 rows of pink kernels.

In the late 1800s the demand for maize suited for human consumption and export purposes (such as Legkorábbi Székely) increased, so, following the advice of Sándor Cserháti, the father of Hungarian maize production, many large farms started breeding maize.

In 1880, Lajos Kovács, who worked on Prince Joseph's estate in Alcsút (Hungary), collected breeding stock from Béllye to develop a new variety (Péterfy, 1923a). He gradually developed a thin-stalked, prolific variety with shorter, less conical ears bearing 20–24 kernel rows with a very marketable kernel colour, which flowered 2 weeks earlier. This variety, Alcsúti Pignoletto, could be sown with a row distance of 40 cm and could be reaped and threshed like winter wheat. This variety won a gold medal at the National Maize Exhibition in 1914.



Chutucuno Grande (Source: Thimothy et al., 1961)

Alcsúti pignolettó Sátorhelyi pignolettó Esterházai pignolettó (Source: Villax Ö.-Surányi J., 1932)

Fig. 6. Varieties related to Pignoletto

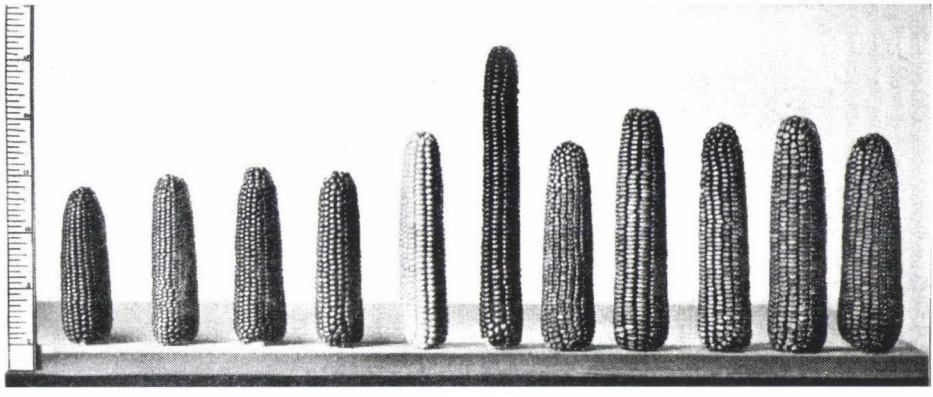


Fig. 7. Hungarian flint and dent varieties (source: Surányi, 1938)
From left: Eszterházi Pignoletto, Bánkúti Early Flint, Sátorhelyi Pignoletto, Putyi, Lovászpatonai Early White, Pennsylvania 8-row, Bánkúti Early Dent, Pettendi Golden Flood, Fleischmann Early Yellow Dent, Lovászpatonai Yellow Dent, Bánkúti (late) Yellow Dent

Balázs Barna improved Alcsúti Pignoletto for prolificacy (Barna, 1904). One mother plant from this stock, which had eight ears, was multiplied in the Crop Production Institute in Mosonmagyaróvár by Emil Grábner, and by 1908 a high-yielding, early maturing variety with 4–6 ears had been developed (Grábner, 1913). Some of this breeding stock was passed on to Lőrinc Falb, a breeder working on the estate of Imre Károlyi in Zalaszentgrót, Hungary (Fabricius, 1921), and in 1923 a new variety was registered under the name of Zalaszentgróti Prolific Pignoletto. A cross between the 8-eared Alcsúti Pignoletto and a related variety, Bánkúti (late) Dent led to the selection of a further high-yielding variety, Esterházai Pignoletto, which had considerably larger ears and long, hard, pink kernels (Grábner, 1913). From 1920 onwards breeding was continued by Imre Günther.

László Baross, who worked on Prince Joseph's estate in Bánkút (Hungary), also selected material from the Béllye Pignoletto breeding stock in 1894 (Péterfy, 1923b). This material was crossed with Queen of the Prairie, Bristol, Mastodon and an unidentified white dent. To start with, Baross carried out mass selection for the desired phenotype, and later adopted the pedigree breeding method, finally producing the variety Bánkúti Kései Lófogú (Bánkúti Late Dent), which had large ears 20–22 cm in length with 20–24 rows of exceedingly long, hard dent kernels, and by 1910 had become popular throughout Central Europe, winning a gold medal at the National Maize Exhibition in 1914. During its approximately 50-year lifespan this was one of the most popular varieties, topping the sales list for many years. Between 1938 and 1962 it was still sown on 4–5% of the growing area. For food industry and export purposes, however, considerably earlier maturing varieties with similar high yields were required. The much earlier Bánkúti Korai Lófogú (Bánkúti Early Dent) was developed in 1908 from a cross between Bánkúti Late Dent and an early variety from Banest, Romania, while a cross between Bánkúti Late Dent and Alcsúti Pignoletto led to the development of Bánkúti Early Flint. Baross was also interested in silage maize, and crossed Bánkúti Early Flint with the variety King Philip to produce the variety Bánkúti Siló (Bánkúti Silage) registered in 1926. Unfortunately no data are available on the spread of the early Bánkúti varieties.

Varieties based on Cinquantino, which matured earlier and had slightly softer kernels, were grown chiefly on small farms for home use, while the later-maturing, harder-grained Pignoletto type of varieties were grown on large farms, primarily for export purposes. Between the two world wars, and especially after the second world war, the structure of human nutrition changed, however. As less and less maize was used to make porridge for human consumption, the higher yielding, later maturing, well-adapted dent varieties gradually ousted early maturing varieties from production. Very few hard flint maize varieties were still grown when the inbred hybrid era began. For the most part, these were ignored by breeders developing inbred lines. The only exceptions appear to be SzV 293, a line bred by Gabriella Székács from Putyi, and B 125, developed from Bánkúti Early Dent by Ferenc Beke, both of which were used as parents in cultivated hybrids.

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ISSN 0238-0161

Printed in Hungary
PXP, Budapest

Acta Agronomica Hungarica

An International Multidisciplinary Journal in Agricultural Science

VOLUME 53, NUMBER 2, 2005

Akadémiai Kiadó, Budapest

MEMBER OF WOLTERS KLUWER GROUP

ACTA AGRONOMICA HUNG. AAHUEX 53 (2) 119–239 (2005) HU ISSN 0238-0161

ACTA AGRONOMICA HUNGARICA

A QUARTERLY OF THE HUNGARIAN ACADEMY OF SCIENCES

Acta Agronomica Hungarica publishes papers in English on agronomical subjects, mostly on basic research

Acta Agronomica Hungarica is published in yearly volumes of four issues by

AKADÉMIAI KIADÓ, Budapest, Hungary

Acta Agronomica Hungarica is abstracted/indexed in Biological Abstracts, BIOSIS Previews, CAB Abstracts, Chemical Abstracts, GEOBASE, Global Health, Index Veterinarius, International Bibliographics IBZ and IBR

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Acta Agronomica Hungarica
Agricultural Research Institute of the
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The Agricultural Research Institute of the Hungarian Academy of Sciences contributes financially to the publication of *Acta Agronomica Hungarica.*

AAgr 53 (2005) 2

ACTA AGRONOMICA HUNGARICA

AN INTERNATIONAL MULTIDISCIPLINARY JOURNAL IN AGRICULTURAL SCIENCE

VOLUME 53, NUMBER 2, 2005

AKADÉMIAI KIADÓ, BUDAPEST

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Acta Agronomica Hungarica, 53(2), pp. 119–131 (2005)

RESPONSES OF MAIZE (*ZEA MAYS* L.) HYBRIDS TO SOWING DATE, N FERTILISER AND PLANT DENSITY IN DIFFERENT YEARS

Z. BERZSENYI and D. Q. LAP

AGRICULTURAL RESEARCH INSTITUTE OF THE HUNGARIAN ACADEMY OF SCIENCES, MARTONVÁSÁR, HUNGARY

Received: 21 April, 2005; accepted: 9 June, 2005

The responses of Hungarian-bred maize hybrids with different vegetation periods to sowing date, N fertiliser and plant density were studied in small-plot field experiments between 2002 and 2004. The maize grain yield was highest in the early and optimum sowing date treatments (8.563 and 8.325 t ha^{-1}) and significantly less in the late and very late treatments (7.908 and 7.279 t ha^{-1}). The year had a substantial effect on both the yield and the grain moisture content.

In a long-term maize monoculture experiment set up in 1961, the N fertiliser responses of 6 maize hybrids with different vegetation periods were investigated. Averaged over the years 2002 and 2004 the maize grain yields in the N treatments were as follows (t ha^{-1}): N_0: 4.780, N_{80}: 7.479, N_{160}: 8.577, N_{240}: 8.226. The grain yield and yield stability of maize were greatest at a N rate of 160 kg ha^{-1}. The yield response was similar in both years, but the year had a considerable effect on the yield level. The N supplies to maize plants during the vegetation period could be well characterised using a SPAD 502 chlorophyll meter in the R3 phenological stage (18–22 days after silking).

The plant density responses of maize hybrids were described by fitting a quadratic function to the data of 19–22 hybrids in the years 2002–2004. The optimum plant density averaged over the hybrids was between 67,483 and 70,161 plants ha^{-1}. The maximum yield associated with optimum plant density was 7.978 t ha^{-1} in 2002, 6.60 t ha^{-1} in 2003 and 9.37 t ha^{-1} in 2004. The annual patterns of plant density responses for the maize hybrids exhibited considerable differences.

Key words: maize, sowing date response, N fertiliser response, plant density response, year effect, long-term experiment

Introduction and literary review

The yield of the maize hybrids grown today (in terms of grain yield per unit area) substantially exceeds that of older hybrids. In the USA the annual yield increase since the first appearance of single cross hybrids has been estimated at 110 kg ha^{-1} (Troyer, 1995). This yield increase can be attributed to genetic gain, agronomic innovation and the genetic × agronomic interaction (Berzsenyi and Győrffy, 1995; Tollenaar and Lee, 2002). The superiority of new hybrids is partly associated with their ability to tolerate and take advantage of higher plant density (Carlone and Russell, 1987; Duvick, 1997). Plant density is the agronomic factor that has changed to the greatest extent in recent decades. The increase in maize yields is related to the improved tolerance of modern hybrids to various biotic and abiotic stress factors, thanks partly to breeding for greater yield stability (Tollenaar and Wu, 1999).

Modern hybrids utilise resources more efficiently than older genotypes. Duvick (1992) reported that newer hybrids gave greater yields than old hybrids under unfavourable and favourable conditions alike. In experiments carried out by Carlone and Russell (1987) single cross hybrids developed in the 1980s had better yield responses to higher rates of nitrogen fertiliser than those developed in the 1960s and 1970s. Environmental changes associated with different sowing dates (sunshine, temperature) have a modifying effect on the growth and development of maize plants. Each hybrid has an optimum sowing date, and the greater the deviation from this optimum (early or late sowing), the greater the yield loss (Sárvári and Futó, 2000; Berzsenyi and Lap, 2001).

A large volume of data is available in the Hungarian literature on the N fertiliser responses (e.g. Győrffy, 1979; Menyhért, 1985; Nagy, 1988; Berzsenyi, 1993; Sárvári, 1995a; Széll et al., 2003; Berzsenyi and Lap, 2003b) and plant density responses (e.g. Győrffy, 1979; Berzsenyi et al., 1994; Nagy, 1995; Sárvári, 1995b; Berzsenyi and Lap, 2003a) of maize hybrids in various ecological regions. A review of the genetic gain in Hungarian maize breeding and the results of maize agronomy research can be found in the proceedings of the jubilee session held to celebrate the 50th anniversary of the first Hungarian maize hybrid (Marton and Árendás, 2003).

Each genotype has a well-defined optimum range, in terms of sowing date, plant density and nutrients, for the achievement of maximum yield. The environmental and agronomic responses of maize hybrids determine their adaptability and influence improvements in maize production through agronomy and breeding. The importance of this research is given by the need to substantially increase the efficiency of maize production. An understanding of the environmental and agronomic responses of maize hybrids is fundamental to improving efficiency.

The aim of the present research was to determine the optimum range of agronomic responses and the predicted yield levels, and to investigate the factors responsible for differences in yields. In small-plot field experiments set up in 2002–2004 investigations were made on the responses of Hungarian-bred maize hybrids with various vegetation periods to sowing date, N fertiliser and plant density.

Materials and methods

The ecological and agronomic responses of maize hybrids were examined in small-plot field experiments set up in the institute nursery in Martonvásár between 2002 and 2004. The soil of the experimental area is a humus-rich loam of the chernozem type with forest residues, mildly acidic in the ploughed layer, poorly supplied with available phosphorus, but with good supplies of potassium.

The weather in the three experimental years differed considerably, being most favourable in 2004 and least favourable in 2003. Rainfall during the vegetation period (Apr.–Sep.) amounted to 300 mm in 2004, close to the 30-year mean (312 mm), and was favourably distributed. The mean temperature during the growing season was 16.8°C, a little lower than the 30-year mean (17.7°C). The number of very hot days (daily maximum of over 30°C) was lower in 2004 (25) than in the other years. In 2003, the worst year for maize, the total rainfall in the vegetation period

was 178 mm, the mean temperature 18.8°C and the number of very hot days 70. In 2002 the total rainfall in the growing season was 326 mm, above the 30-year mean, but its distribution was unfavourable. During flowering, from mid-June to early July, there was only 20 mm rainfall. The mean temperature during the vegetation period (18.4°C) exceeded the 30-year mean, with 43 very hot days (29 of them during the critical period for kernel set).

Mouldboard ploughing was carried out in autumn, followed by conventional seedbed preparation (disc, cultivator, harrow) in the spring. Weed control consisted of the presowing application of butilate, and preemergence treatment with acetochlor and atrazine. Herbicide treatment was supplemented by manual hoeing as required. A Wintersteiger plot-sized seed drill was used for sowing and a Bourgoin plot combine for harvesting.

Treatments

The effect of sowing date on the grain yield and grain moisture (%) at harvest of Hungarian-bred hybrids was analysed in a bifactorial split-plot experiment set up in three replications in 2002 and 2003 and four replications in 2004, with sowing dates in the main plots and maize hybrids in the subplots. The subplot size was 2.8 × 6 m = 16.8 m² and the spacing 70 × 20 cm. The four sowing dates (early, optimum, late and very late) represented realistic sowing dates in farm practice. The sowing dates were Apr. 18, 29, May 8, 17 in 2002, Apr. 16, 25, May 6, 16 in 2003 and Apr. 20, 29, May 10, 20 in 2004. The responses of 6–7 hybrids were tested each year: Mv TC 277 (FAO 310) and Borbála (FAO 330) in 2002 and 2003, Szegedi TC 352 (FAO 320) in 2004, Hunor (FAO 370) in 2003 and 2004, Debreceni SC 377 (FAO 340), Maraton (FAO 450) and Szegedi 428 (FAO 430) in 2002 and 2003, Szegedi SC 463 (FAO 450) in 2004 and Vilma (FAO 510) in 2002–2004.

The N fertiliser response of maize hybrids was analysed in a long-term maize monoculture set up in 1961. In the split-plot design with four replications the N treatment was in the main plot and the maize hybrid in the subplot. The N fertiliser rates were: 0, 80, 160 and 240 kg ha^{-1} (designated as: N_0, N_{80}, N_{160}, N_{240}). All treatments received 160 kg ha^{-1} each of P and K fertiliser. The size of the subplot was 6.4 m². The experiment was set up in 2002 and 2004 with six Martonvásár-bred maize hybrids from different maturity groups: Mv TC 277 (FAO 310), Norma SC (FAO 380), Mv 355 DMSC (FAO 390), Gazda MTC, Maraton and Mv 444 (FAO 450).

The effect of plant density on the grain yield of maize hybrids was evaluated at 8 different plant densities at 10,000 plant ha^{-1} intervals in the 30,000–100,000 plant ha^{-1} range. The experiment was set up in the institute nursery in a split-plot design (main plot: plant density, subplot: hybrid) in four replications, with a subplot size of 5.6 m². The plant density responses of 19–22 hybrids were investigated each year from 2002–2004. The results were evaluated each year over the average of the hybrids, while the year effect on plant density response was compared for five hybrids: Mv TC 277, Debreceni SC 377, Hunor, Mv 355 DMSC and Maraton.

Data collection

The tasselling and silking dates were recorded, and the plant number, ear number, ratio of barren and lodged plants, and level of smut (*Ustilago maydis, Sorosporium holci-sorghi*) infection were determined prior to harvest. Five sample ears were taken from each experimental plot for the determination of yield components and yield quality.

The N supplies to maize plants in the various treatments were analysed using a portable SPAD-502 chlorophyll meter that estimated the chlorophyll content (green colour intensity) based on the light transmission at 440 nm and 670 nm from a 2 × 3 mm leaf area. A significant linear correlation was found between the SPAD units and the extractable chlorophyll concentration of the maize leaf (Dwyer et al., 1991). Other authors proved a close correlation between the SPAD units and the plant N supplies (Schepers et al., 1992). Measurements were taken on the leaf next to the ear after silking, on 30 plants per plot. At harvest the grain yield and moisture content were recorded, and the yield was calculated for a moisture content of 15%.

Analysis of variance and regression analysis

In the sowing date and N fertiliser experiments, the effect of the treatments on maize grain yields was first evaluated for each year separately using two-factor analysis of variance according to Sváb (1973), followed by combined analysis of variance on the data of two or three experimental years, as described by Gomez and Gomez (1984). The plant density responses of maize hybrids were evaluated using regression analysis. A quadratic function ($Y = a + bx - cx^2$) was fitted to the data, which were processed using the statistical programs MSTAT-C and SPSS 11.0.

Results

Sowing date response of maize hybrids

The results of two-factor analysis of variance indicated that the sowing date had a significant effect on the maize grain yield, at the 5% level of probability in 2002 and at the 0.1% level in 2003 and 2004. It can be seen from Table 1 that yields were highest in all the years in the optimum and early sowing date treatments, significantly declining when sowing was delayed by 10 or 20 days. Combined analysis of variance was carried out on the four hybrids tested in all three years (Mv 277, Debreceni SC 377, Maraton, Vilma). The maize grain yield was highest in the early and optimum sowing date treatments (8.563 and 8.325 t ha^{-1}) and significantly lower in the late and very late treatments (7.908 and 7.279 t ha^{-1}).

The grain yields recorded for the maize hybrids, averaged over sowing dates, are presented in Table 2. Analysis of variance revealed that the differences between the yields of the various hybrids was significant at the 0.1% level in all three years, but differed from one year to the next. Averaged over sowing dates and hybrids, the highest yield was obtained in 2004 (8.677 t ha^{-1}), followed by 2002 (7.918 t ha^{-1}), with the lowest yield in 2003 (6.411 t ha^{-1}). The sowing date × hybrid interaction was significant in 2002 and 2003, but the deviation in mean squares (MQ) showed that the magnitude of this interaction was substantially smaller than that of the main effects.

On the basis of two-factor analysis of variance, the effect of the sowing date on the grain moisture content at harvest was significant at the 0. 1% level in all three years. Averaged over the years, the moisture content was lowest in the early and optimum sowing date treatments (17.37 and 17.99%), being significantly higher in the late and very late treatments (19.35 and 21.54%) (Table 3). The grain moisture content and the difference between sowing dates were considerably influenced by the year. Averaged over sowing dates and hybrids, the grain moisture at harvest was lowest in 2002 (16.93%), followed by 2003 (18.04%), with the highest value in 2004 (21.04%).

The grain moisture at harvest, averaged over the sowing dates, is presented in Table 4, which shows that the grain moisture tended to be higher in hybrids with longer vegetation periods. The sowing date × hybrid interaction was not significant in 2002 or 2003. In 2004 the interaction was significant at the 5% level, but the MQ value was extremely low compared to those of the main effects (sowing date, hybrid).

Table 1
Effect of sowing date on maize grain yields in 2002–2004

Sowing date	Maize grain yield t ha^{-1}			
	2002	2003	2004	2002–2004*
Early	7.653 ab	7.801 a	9.206 a	8.563 a
Optimum	8.536 a	6.413 b	9.312 a	8.325 a
Late	8.460 a	5.957 c	8.183 b	7.908 b
Very late	7.022 b	5.471 d	8.005 b	7.279 c

*Based on the data of four hybrids (Mv 277, Debreceni SC 377, Maraton, Vilma); treatments designated with the same letter within each column did not differ significantly according to Duncan's Multiple Range Test.

Table 2
Grain yields (t ha^{-1}) obtained for the maize hybrids tested in the sowing date experiment (2002–2004)

2002		2003		2004	
Maize hybrid	Grain yield	Maize hybrid	Grain yield	Maize hybrid	Grain yield
Mv 277	8.413 b	Mv 277	5.726 e	Mv 277	8.776 b
Borbála	6.804 d	Borbála	5.961 de	Szegedi SC 352	8.719 b
Debreceni SC 377	8.152 b	Debreceni SC 377	6.639 b	Debreceni SC 377	8.734 b
–	–	Hunor	6.515 bc	Hunor	8.825 b
Maraton	7.994 b	Maraton	7.524 a	Maraton	8.867 b
Szegedi 428	7.303 c	Szegedi 428	6.178 cd	Szegedi SC 463	7.179 c
Vilma	8.841 a	Vilma	6.332 bc	Vilma	9.636 a

Treatments designated with the same letter within each column did not differ significantly according to Duncan's Multiple Range Test.

Table 3
Effect of sowing date on the maize grain moisture content at harvest (2002–2004)

Sowing date	Grain moisture content (%)			
	2002	2003	2004	2002–2004 Mean*
Early	15.99 b	16.69 b	19.01 d	17.37 d
Optimum	16.39 b	16.91 b	19.72 c	17.99 c
Late	16.62 b	18.90 a	21.25 b	19.35 b
Very late	18.72 a	19.67 a	24.25 a	21.54 a

*Based on the data of four hybrids (Mv 277, Debreceni SC 377, Maraton, Vilma); Treatments designated with the same letter within each column did not differ significantly according to Duncan's Multiple Range Test.

Table 4
Grain moisture content (%) of maize hybrids at harvest tested in the sowing date experiment (2002–2004)

2002		2003		2004	
Maize hybrid	Moisture	Maize hybrid	Moisture	Maize hybrid	Moisture
Mv 277	16.47 cd	Mv 277	17.06 c	Mv 277	19.72 e
Borbála	16.29 d	Borbála	17.10 c	Szegedi SC 352	20.15 de
Debreceni SC 377	16.85 c	Debreceni SC 377	19.02 a	Debreceni SC 377	21.46 c
–	–	Hunor	17.27 bc	Hunor	20.44 d
Maraton	17.32 b	Maraton	18.58 a	Maraton	22.26 b
Szegedi 428	16.57 cd	Szegedi 428	18.19 ab	Szegedi SC 463	20.58 d
Vilma	18.08 a	Vilma	19.10 a	Vilma	22.78 a

Treatments designated with the same letter within each column did not differ significantly according to Duncan's Multiple Range Test.

Late sowing leads to a shortening of the vegetative growth period of maize, as is clearly revealed by the reduction in the number of days from sowing to 50% tasselling and 50% silking. In 2004 the number of days from sowing to 50% tasselling in the various sowing date treatments was 83.14 (early), 77.64 (optimum), 73.18 (late) and 69.29 (very late). In the same order these figures were 85.04, 80.07, 75.29 and 71.57 for days to 50% silking. The other major effect of the sowing date was the lengthening of the maturity period in the late and very late treatments.

Effect of N fertilisation on the grain yield and N fertiliser responses of maize hybrids in a long-term experiment

Two-factor analysis of variance revealed that the effect of N fertilisation on the grain yields of maize hybrids was significant at the 0.1% level in both 2002 and 2004. The effect of the hybrid was also significant at the 0.1% level, but the MQ values showed the effect of N fertilisation to be more than twenty times greater than that of the hybrid. The N fertilisation × hybrid effect was not significant in 2002, i.e. the N fertiliser responses of the hybrids did not differ at different N rates. In 2004 this interaction was significant.

The yield-increasing effect of N fertilisation at each N rate, averaged over the hybrids, is shown in Table 5. The maize grain yield can be seen to rise consistently with the N fertiliser rate up to a rate of 160 kg ha^{-1} N, after which the change was not significant. The yield response was similar in both years, but in 2004, due to the better weather, the yield level was higher (7.991 t ha^{-1}) than in 2002 (6.539 t ha^{-1}). The N fertiliser responses of the six tested hybrids in 2002 and 2004 are illustrated in Figure 1, which shows that the yields of hybrids with longer vegetation periods were considerably higher in 2004 than in 2002.

As the result of N fertilisation there was a significant reduction in the time from sowing to silking in both years, compared with the unfertilised control plots. Averaged over the hybrids, the number of days from sowing to silking was as follows: N_0: 77.7, N_{80}: 73.9, N_{160}: 72.8, N_{240}: 73.3 in 2002, and N_0: 82.5, N_{80}: 80.2, N_{160}: 79.8, N_{240}: 79.7 in 2004.

Table 5
Effect of N fertiliser on the grain yield of maize in a long-term monoculture experiment (2002–2004, mean of 9 hybrids)

N fertiliser rate, kg ha^{-1}	Maize grain yield, t ha^{-1}	
	2002	2004
0	4.083 c	5.476 c
80	6.656 b	8.302 b
160	7.839 a	9.315 a
240	7.578 a	8.873 ab

Treatments designated with the same letter within each column did not differ significantly according to Duncan's Multiple Range Test.

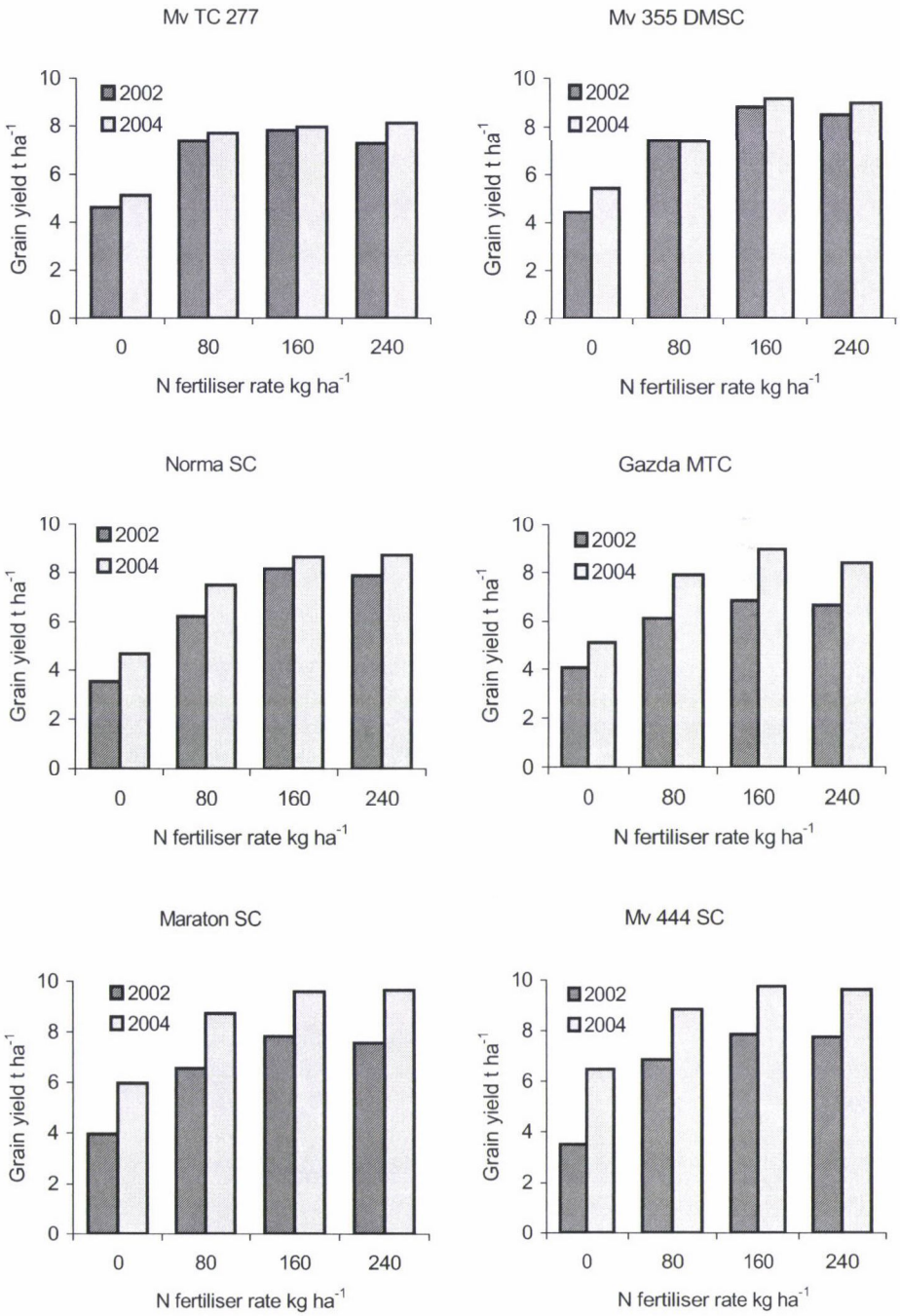

Fig. 1. N fertiliser responses of maize hybrids in 2002 and 2004

The N supply level of the maize plants during the vegetation period could be well characterised using a SPAD 502 chlorophyll meter. Averaged over the maize hybrids, the N fertiliser effect in the R3 phenological stage (18–22 days after silking) was characterised by the following SPAD units: N_0: 31.1, N_{80}: 47.0, N_{160}: 52.1, N_{240}: 52.3 in 2002, and N_0: 33.4, N_{80}: 46.3, N_{160}: 50.4, N_{240}: 52.0 in 2004. It can be seen that the SPAD units increased significantly up to a rate of 160 kg N ha^{-1}, after which the changes were no longer significant. There were also significant differences between the SPAD units for the individual hybrids. A close correlation was found between the maize grain yield and the SPAD units.

The effect of N fertilisation on the grain protein content was analysed in 2004. Analysis of variance showed this effect to be significant at the 0.1% level. Averaged over the maize hybrids, the grain protein content in the various N treatments was as follows: N_0: 6.099, N_{80}: 7.092, N_{160}: 7.935, N_{240}: 7.946%. There was thus a significant increase in the grain protein content up to a rate of 160 kg ha^{-1}, after which the change was not significant. Although the grain protein content differed significantly for the tested hybrids, the N fertilisation × hybrid interaction was not significant.

It can be concluded from the above that the effect of N fertilisation on the yield responses of maize hybrids can be satisfactorily determined from the N supplies to the plants (SPAD units), the number of days to silking and the grain protein content.

Plant density response of maize hybrids

The plant density responses of 19–22 hybrids were studied each year between 2002 and 2004 at eight plant densities, increasing by intervals of 10^3 plants ha^{-1} over the range 30–100 thousand plants ha^{-1}. A quadratic function was fitted to the data. The F-probe on the functions and the t-probe on the function parameters were significant at the 0.1% level in all cases. The three different years allowed the plant density responses of the hybrids to be compared under various rainfall supply conditions.

Quadratic functions fitted to the annual data of five maize hybrids with different vegetation periods and to the mean annual data of 19–22 hybrids are presented in Figure 2. The plant density responses averaged over the hybrids provide a clear illustration of the substantial differences from year to year in the yield levels. The highest maize yields were recorded in 2004 (8.594 t ha^{-1}) and the lowest in 2003 (5.949 t ha^{-1}), with an intermediate yield level in 2002 (7.395 t ha^{-1}). Averaged over the hybrids the pattern of plant density response was similar in all three years, so there was little difference in the optimum plant densities per hectare calculated from the functions each year: 68,710 in 2002, 67,483 in 2003 and 70,161 in 2004. The maximum grain yield associated with optimum plant density differed substantially, however, in these years: 7.978 t ha^{-1} in 2002, 6.60 t ha^{-1} in 2003 and 9.37 t ha^{-1} in 2004.

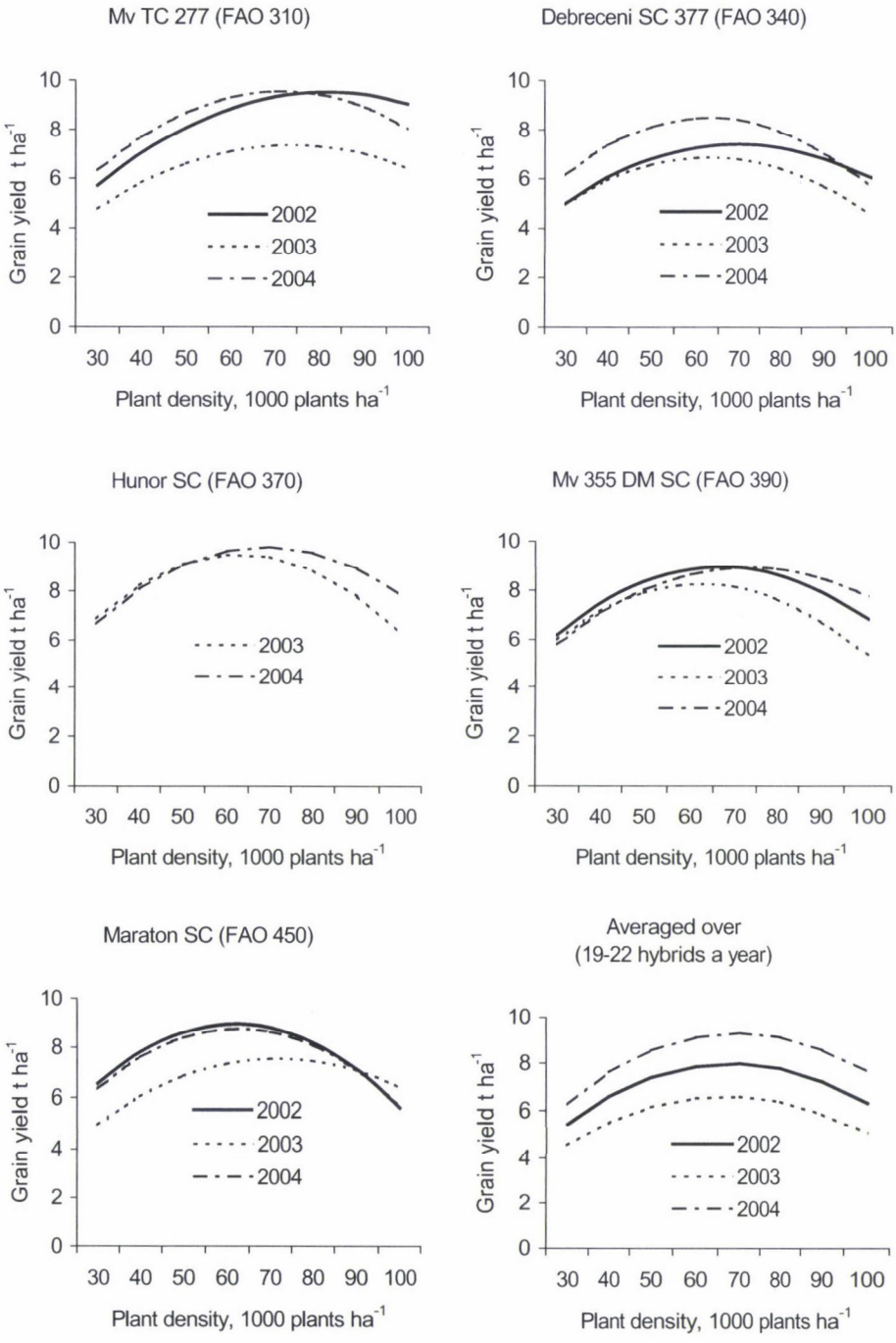

Fig. 2. Plant density responses of maize hybrids between 2002 and 2004

The annual patterns for the plant density responses of individual hybrids differed considerably, allowing hybrid-specific plant density responses to be determined for use by the extension service. The parameters of the quadratic functions fitted to the data allow the rising and falling sections of the plant density response curves to be characterised and conclusions to be drawn on the adaptability of the hybrids in different environments.

Averaged over the hybrids tested each year, the linear regression parameter "b" of the quadratic function was greatest (0.2665) in the most favourable year, 2004, moderate (0.231) in 2002 and smallest (0.1977) in the dry year of 2003. Averaged over the hybrids, the value of the second order regression parameter "c" exhibited less difference between the years (ranging from –0.0015 to –0.0019). Yield data calculated for the lowest plant density, 30,000 plants ha^{-1}, averaged over the hybrids, differed considerably between the years, being 5.42 t ha^{-1} in 2002, 4.59 t ha^{-1} in 2003 and 6.27 t ha^{-1} in 2004.

Discussion

The agronomic responses of Hungarian-bred maize hybrids with different vegetation periods were examined between 2002 and 2004. The sowing date experiment created different ecological conditions through changes in the temperature, intensity of illumination and photoperiod duration. The significance of the sowing date is proved by the fact that the effect of sowing date on the maize grain yield and on the grain moisture content at harvest was significant in all the years. The highest grain yield was recorded in the early and optimum sowing date treatments, with significant reductions in the late and very late treatments. It is known from earlier studies that in dry years very late sowing may lead to yield losses as great as 30–40% (Berzsenyi and Lap, 2001). The yield reductions observed after late and very late sowing can be attributed to unfavourable changes in the environment, leading to lower values of growth parameters, especially during the reproductive phase (Berzsenyi et al., 1998). The correlation between the sowing date and the grain moisture at harvest (%) was the opposite of that between the sowing date and the yield. After late or very late sowing the grain moisture at harvest increased consistently, but to different extents for each hybrid.

In a long-term N fertilisation experiment set up over 40 years ago, the various N supply levels represent different ecological conditions. Various evaluation methods have all demonstrated that the maize grain yield and the N fertiliser response are substantially influenced by the level of N fertilisation, the year and the maize hybrid. The maize grain yield gradually increased with the N fertiliser rate up to the N_{160} level, after which the change was not significant. Under the given experimental conditions the highest yield was achieved with 160 kg N ha^{-1}. The N fertiliser responses of the maize hybrids exhibited great annual fluctuation, which, under the conditions of the long-term experiment,

could be attributed mainly to year effects related to rainfall supplies. The SPAD 502 chlorophyll meter was found to be a useful instrument for estimations of the relative chlorophyll concentration of the leaf and for the characterisation of the N supplies to maize plants during the vegetation period. The importance of this is indicated by the increasing emphasis given in crop production research to the continuous monitoring of the plant stand and of individual plants, and to ecophysiological measurements on plant responses.

In the plant density experiment changes in the spacing altered the microenvironment of the plants and increased competition for major ecological factors (illumination, water, nutrients). The three different years allowed the plant density responses of the maize hybrids to be compared in environments with different rainfall supplies. The parameters of the quadratic function fitted to the data provided an accurate characterisation of the plant density responses. The annual patterns of these responses differed considerably for the individual hybrids, allowing hybrid-specific plant density responses to be determined. The optimum plant density, averaged over the hybrids, was similar in the three years (ranging from 67,483 to 70,161 plants ha^{-1}), while the maximum grain yield differed substantially (6.60 t ha^{-1} in the least favourable year, 2003, and 9.37 t ha^{-1} in the best year, 2004). The evaluation of data for the previous 22 years (1981–2002), however, indicates that the optimum plant density may vary considerably in different years (Berzsenyi and Lap, 2003a). Under the given experimental conditions the yield was the most stable at a plant density of 60,000 plants ha^{-1}. High plant density has an unfavourable effect on yield stability, because of yield losses due to missing plants and uneven emergence (poorly compensated for by the increased yield of the surrounding plants), greater variability per plant (associated with decreased resource use efficiency) and the higher ratio of barren plants (resulting from the increased pollen-to-silking interval) (Berzsenyi et al., 1994; Tokatlidis and Koutroubas, 2004).

Acknowledgements

This research was funded by the NKFP maize consortium (No. 4/008/2002).

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Acta Agronomica Hungarica, 53(2), pp. 133–141 (2005)

EFFECT OF THE YEAR ON THE VEGETATIVE AND GENERATIVE PHASES IN THE GROWING PERIOD OF MAIZE

L. C. MARTON, T. SZUNDY and I. PÓK

AGRICULTURAL RESEARCH INSTITUTE OF THE HUNGARIAN ACADEMY OF SCIENCES, MARTONVÁSÁR, HUNGARY

Received: 10 June, 2005; accepted: 20 July, 2005

The evaluation of an 8 × 8 diallel cross revealed that the mean length of the growing period was the same for inbred lines and their hybrids. However, the vegetative phase was 7–8% shorter and the generative phase 7–8% longer for the hybrids than for the inbred lines.

The generative phase of the growing period, unlike the vegetative phase, proved to be extremely variable. Under dry conditions the length of the generative phase was negatively correlated with the length of the vegetative phase. The later a genotype flowered the less time remained for grain filling, due to the stress which curtailed the growing period. The shorter generative phase of late-flowering hybrids was the reason for the weakening of the growing period–yield correlation under dry conditions. This vulnerability of late-flowering hybrids makes selection for stress tolerance particularly important.

Key words: maize, *Zea mays*, growing period, generative phase, vegetative phase

Introduction

The growing period is the time from sowing to maturity (Jugenheimer, 1958), expressed in terms of days or heat units. A knowledge of the growing period is important not only for predicting maturity and harvest date, but also due to the close correlation between growing period and yield potential.

The determination of the growing period is complicated by the difficulty experienced when trying to define or measure maturity. Originally the maturity date was estimated from the formation of the dent shape on the kernel tip or from the yellowing of the husks (Wallace and Bressman, 1925; Alberts, 1926). Physiological maturity was estimated from the maximum thousand-kernel mass (Aldrich, 1943; Shaw and Loomis, 1950). On the basis of experiments carried out in Iowa, Shaw and Thom (1951) found that the period from silking to maturity was a constant 51 days irrespective of the genotype, year and environment. As the silking–maturity period proved to be stable, many authors recommended determining the growing period in terms of the number of days from sowing to silking (van Eynatten, 1957; Mohamed, 1959; Giesbrecht, 1960a, b) or the active heat sum accumulated during this period (Gilmore and Rogers, 1958; Tsotsis, 1959). Chase (1964) and Chase and Kanada (1967) found that the date of silking was extremely important not only in terms of the growing period, but also as regards yield potential. The yield of hybrids with the same grain moisture content at harvest was in close positive correlation with the date of silking.

0238–0161/$ 20.00

Derieux and Bonhomme (1982) underlined the fact that silking is a trait that can be easily scored and precisely determined, having a high h^2 value. The flowering dates of individual genotypes can be predicted with even greater certainty if heat sums are used in the calculation rather than days. A delay in silking can be expected in the case of stress, with an increase in the interval between tasselling and silking. In extreme cases this may be sufficient to prevent fertilisation (Troyer and Brown, 1976; Troyer, 1983; Russell, 1985a, b).

The genetic variability of the flowering date in maize breeding stocks is extremely wide, allowing maize, which is of subtropical origin, to be grown successfully from the tropics to a latitude of 55° (Pintér et al., 1995; Marton, 2000).

Although the early reports on the constant length of the generative period were contradicted by Dessureaux et al. (1948), Hallauer and Russell (1962) and Hanway (1963), these authors found very little variability between the hybrids. Similar conclusions were drawn by Gunn and Christensen (1965) and Hillson and Penny (1965). Shaver (1986), however, reported that the generative phase of the variety Cuzco was 150 days in California, compared with 60 days for local hybrids, while Boyle et al. (1979) stated that later hybrids had longer generative phases.

Zametra (1983) carried out selection for a lengthening of the generative phase, which led to a simultaneous increase in the length of the vegetative phase. Diaz and Rivera (1977) also observed that there was a positive relationship between the vegetative and generative phases in maize hybrids. The generative phase was found to have a greater effect on the yield than the vegetative phase.

The fact that early maturing had a negative effect on the yield was attributed by Frohner (1978) to the shortness of the generative phase, which was in close correlation with the vegetative phase.

A close positive correlation between flowering date and the length of the generative phase was established by Carter and Poneleit (1973) and Farhatullah (1989). Similar results were reported by Hadi (1982). In contrast, however, Hillson and Penny (1965) stated that early-flowering hybrids required the longest time from flowering to physiological maturity, though these experiments were made on lines with very similar flowering periods and their hybrids.

When evaluating hybrids from different periods, Daynard and Kannenberg (1976) found that modern hybrids had a longer grain filling period, allowing them to give higher yields.

Diverse results were reported on the lengths of various phases of the growing period and on the mutual ratios of these phases. The majority of observations indicated that the vegetative phase was more variable than the generative phase, and that the two phases were proportionate to each other in length.

The present experiments used an 8×8 diallel system to study the correlation between the length of various phases in the growing period of maize and their mutual ratios under Hungarian ecological conditions in different years.

Materials and methods

The tests were made on eight inbred lines with different agronomic traits and genetic backgrounds (Table 1), and on crosses carried out according to a diallel scheme. As these eight lines differed substantially in earliness and genetic background, they provided an excellent opportunity for tests on the determination of growing period.

The experiments were carried out in the Lászlópuszta nursery of the Martonvásár institute over a two-year period. The first year was relatively favourable for maize production, while the second was dry and unfavourable. The experiments were laid out in two replications in a split plot design, with a stand density of 70,000 plants/ha. The genotypes were grouped according to their heterozygosity level (line, hybrid), but were sown at random within the groups. The following traits were evaluated:

1. Number of days from sowing to 50% silking. Plants were regarded as flowering if the silks had emerged from the husks by at least 1 cm.

2. Grain filling period: the number of days from silking to physiological maturity – development of maximum thousand-kernel mass.

Samples were taken from all the genotypes from 30–35 days after silking for the determination of physiological maturity. Each year 7 samplings were made at 10-day intervals. At each sampling date three ears were removed from each replication of each genotype, after which the kernels from the three ears in each sample were mixed, and 2×100 kernels from each replication were dried to constant weight in a drying cabinet. A second degree polynomial equation ($y = ax^2 + bx + c$) was fitted to the grain moisture and thousand kernel-mass data for each replication (Sváb, 1981).

Table 1
Characteristics of the inbred lines tested

No.	Line	Year of development	Origin	Kernel type	Tasselling (days) F2=0
1	A 654	1966	A116*WF9	dent	+ 1
2	Mo 17	1964	187-2*C103	dent	+13
3	Mv 16	1983	FR19*CM105	dent	+ 7
4	HMv 307	1983	P3709MSC	dent	+ 8
5	HMv 2728	1983	Minneapolis	flint	– 7
6	F 2		OP. Lacaune	flint	0
7	CM 174	1970	V3*B14[2]	dent	+ 3
8	HMv 651	1946	Mps	dent	+ 7

Results and discussion

The growing period is divided into two distinct phases by flowering. Considerable differences in the vegetative phase, lasting until silking, were observed among the genotypes. There was a difference of 23 days between the flowering dates of the earliest (HMv 2728; 67.8 days) and the latest (Mo 17; 90.9 days) lines, but only 17 days between the earliest (HMv 2728*F2; 65.87 days) and latest (Mo17*HMv 651; 83.24 days) hybrids. In general the hybrids flowered earlier than the lines (Fig. 1). The heterosis calculated for flowering date generally shortened the vegetative developmental phase of the hybrids by 8–9 %. On the basis of heat sum data, Duvick (1984) also estimated a 9% difference in flowering date between hybrids and their parental lines.

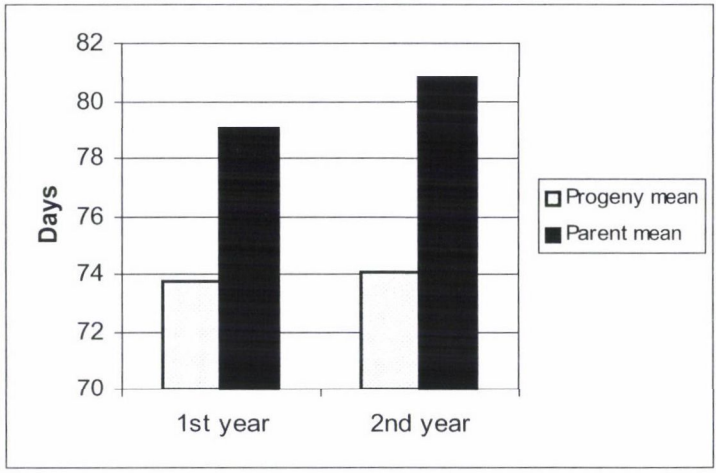

Fig. 1. Silking of inbred lines and their hybrid progeny in terms of days from sowing

The vegetative phase of the growing period exhibited relative stability in the two years. The mean silking date of the hybrid progeny was 0.4 days later and that of the parental lines 1.8 days later in the unfavourable, dry year, compared with the previous year (Fig. 1). All in all it can be said that the silking of the genotypes presented a very similar picture in the two years. A very close correlation ($r = 0.927^{***}$) was found between the silking dates of the genotypes in the two years.

In the first year the generative phase of the growing period was longer for both the parental lines and the hybrids than the vegetative phase. However, while the vegetative phase of development was 6 days shorter for the hybrids than for the parental lines, the generative phase was 6 days longer (Fig. 2).

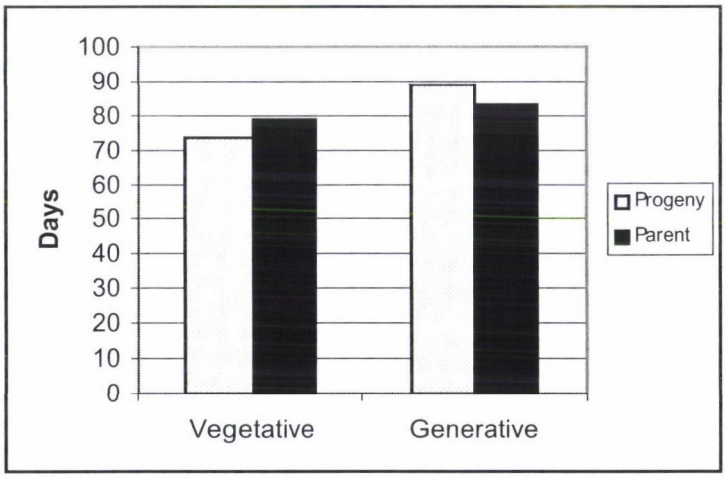

Fig. 2. Length of the vegetative and generative phases in parental lines and their hybrids (Martonvásár, 1st year)

The generative phase of the hybrids was again 6 days longer than that of the parental lines in the second year, and their vegetative phase was again this much shorter (Fig. 3). This opposite change in the length of the generative and vegetative phases in hybrids and parental lines resulted in the total growing period of the hybrids and parental lines being the same in both years.

The data indicate that the equal length of the growing period for hybrids and parental lines was not caused by the different regulation of the length of the generative phase, but by the fact that, due to the unfavourable effect of the weather on both the hybrids and the parental lines, physiological maturity was reached at approximately the same time. This meant that less time remained for the generative phase in late-flowering genotypes and more time in early-flowering plants, from flowering until the end of the life processes. At the same time, the identical growing period for lines and hybrids could be explained by the fact that the lines were compared with their own hybrids.

The shortening of the generative phase in the unfavourable second year was so great that the generative phase of the parental lines (70.25 days) became shorter than their own vegetative phase (80.8 days) (Fig. 3).

Such a drastic reduction in the length of the generative phase is generally caused by cold weather and early frosts in autumn (Miles, 1958; Daynard et al., 1969). In the given years, however, the first frosts did not occur until after the experiment was harvested, so this did not explain the phenomenon. The drought experienced throughout the growing period, especially during the generative phase, combined with extremely high (>30°C) temperatures, appears to have been responsible. In climate chamber experiments conducted by Badu-Apraku et al. (1983) physiological maturity was reached earlier at higher temperatures.

Drought is the reason why late hybrids are unable to achieve their maximum yield potential, and this also explains why physiological maturity (maximum thousand kernel-mass) occurs so early.

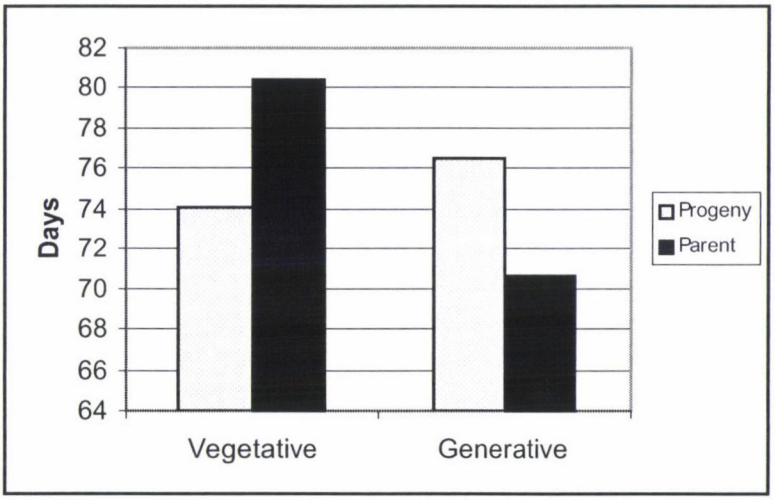

Fig. 3. Length of the vegetative and generative phases in parental lines and their hybrids

The fact that environmental stress has such an intense effect on the generative phase makes it difficult to determine the length of the growing period on the basis of physiological maturity. While a very strong positive correlation was obtained in both years for the length of the vegetative phase, the correlation between the two years was not significant for the generative phase in the hybrids ($r = -0.363^{NS}$). This suggests that, due to differences in the environmental sensitivity of the two phases (vegetative and generative) of the growing period, only the length of the vegetative phase is stable under Hungarian climatic conditions, while that of the generative phase is extremely environment (year, location)-dependent.

The generative phase was found to be constant in experiments carried out by Shaw and Thom (1951), while Hallauer and Russell (1962), Gunn and Christensen (1965) and Hillson and Penny (1965) also found only slight variability. Later reports suggested that the length of the kernel-filling period was proportionate to the number of days to silking, i.e. hybrids with longer growing periods had a longer grain-filling period (Carter and Poneleit, 1973; Diaz and Rivera, 1977; Boyle et al., 1979; Hadi, 1982). The present data, however, indicate that the length of the generative phase is independent of the vegetative phase and exhibits considerable variability.

The correlation between the vegetative and generative phases exhibited great year dependence. In the first year no correlation was found between the two parameters ($r = 0.159^{NS}$). In the second year a close negative correlation ($r = -0.808^{***}$) was found between the length of the vegetative and generative phases, indicating that the longer the vegetative phase of a hybrid, the shorter its generative phase.

The drought affecting the present experiments serves as an explanation for the difference between these results and data from the literature. The high yield potential of late hybrids is no advantage if stress does not allow this potential to be manifested. In the present case the hybrids were only able to achieve 30–50% of their yield potential. This could be attributed in great part to the short generative phase and the premature cessation of life processes.

The close correlation between the length of the vegetative phase and the yield potential was determined under favourable conditions (Chase, 1964; Chase and Kanada, 1967). However, this close positive correlation is only valid if the length of the generative phase is proportionate to that of the vegetative phase. Heat and drought stress cause an unfavourable modification of this ratio, and this is responsible for low yields. Troyer and Brown (1976) and Troyer (1978; 1983) drew attention to the fact that under unfavourable conditions, later flowering populations give lower yields than early flowering genotypes.

The correlation recorded between the length of the vegetative and generative phases in hybrid progeny groups in the second year of the experiment confirmed earlier observations (Fig. 4). Hybrid progeny of the two earliest flowering lines (HMv 2728 and F2) had the shortest vegetative and longest generative phase, while the opposite was true of the hybrid progeny of the latest line, Mo 17, which had the longest vegetative and shortest generative phase.

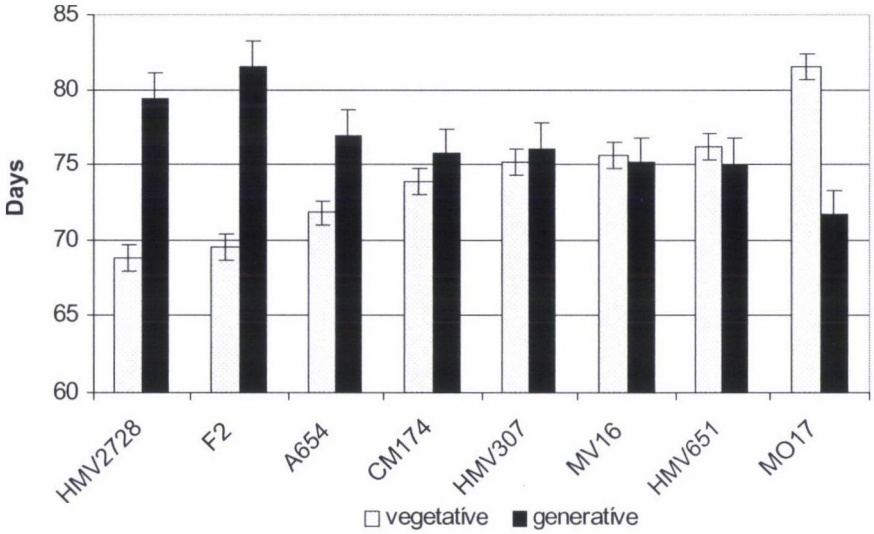

Fig. 4. Vegetative and generative phases of hybrid progeny groups (Martonvásár, 2nd year)

The analysis of hybrid progeny groups revealed that in addition to the basic correlation between flowering time and the generative phase, the stress tolerance of the given genotype also plays a role in determining the length of the generative phase. In the case of F2, and particularly HMv 307, the length of the generative phase was relatively stable, while that of Mv 16, HMv 651 and Mo 17 exhibited a considerable reduction. This stability is proof of the good heat and drought tolerance of HMv 307. Duvick (1984), Russell (1985a, b) and Cavalieri and Smith (1985) also noted that although the flowering date of new hybrids had remained unchanged, they had a longer grain filling period, leading to substantial increases in yield.

In breeding, the effect of both factors – flowering time and stress tolerance – on the yield must be given adequate attention.

In the case of stress, the leaf area may wither as early as August, inducing physiological maturity and thus explaining the negative correlation between silking date and the length of the generative phase. In other words, the later a genotype flowers, the less time it has for grain filling if the vegetation period is curtailed by stress. Due to the exposure of late-flowering genotypes to this danger, it is extremely important to select for stress tolerance, choosing genotypes capable of retaining their foliage for photosynthesis and nutrient transport even under stress conditions. Under Hungarian conditions earlier flowering hybrids are more likely to avoid drought than later flowering ones. Stress tolerance thus plays a much more important role in the growing period and yield stability of the latter.

Acknowledgements

This research was funded by the NKFP maize consortium (No. 4/008/2002).

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Acta Agronomica Hungarica, 53(2), pp. 143–151 (2005)

NEW MAIZE HYBRIDS FROM SZEGED AND THEIR SPECIFIC PRODUCTION TECHNOLOGY

E. SZÉLL, S. SZÉL and L. KÁLMÁN

CEREAL RESEARCH NON-PROFIT COMPANY, SZEGED, HUNGARY

Received: 18 March, 2005; accepted: 6 May, 2005

Four maize hybrids bred at the Cereal Research Non-Profit Company in Szeged were registered at the National Institute for Agricultural Quality Control (OMMI) during the period 2001–2004. The registration of five Szeged hybrids is expected on the territory of the European Union in 2005–2006. The hybrids are accompanied by specific production technological guides for commercial production based on the results of agronomy trials, so that the genetic potential of the hybrids can be utilised in practical farming to the highest possible extent. The specific agronomic traits of hybrids with different vegetation periods and genotypes are investigated.

If a maize hybrid is to be recommended to farmers, it is necessary to know not only its yield potential, but also its yield stability. For this reason, investigations are also made on the effect of soil and climate on the grain yield potential of each hybrid individually.

Key words: registration, plant density response, planting date response, nutrient response, heat and drought tolerance, genotype, production technological guide

Introduction

A great number of retrospective analyses were published in recent decades with the purpose of tracing genetic gain and to make predictions for the future by extrapolating trends (Russell, 1991; Tollenaar and Wu, 1999).

The improvement achieved in genetic yield potential was estimated at 2.5% per year in the USA from the late 1950s to the late 1980s.

The genetic yielding ability of hybrids in Hungary improved at a linear rate of 180–190 kg per year (year effects were damped by a 5-year moving average) from 1965 to the mid-1980s, based on the calculations of Kálmán and Németh (1986). Various factors had dominant and decisive roles in the improvement of the domestic average yield in different eras. In the early 1960s the first hybrids appeared and they spread rapidly by the middle of the decade. In the 1970s–1980s production increased due to the introduction of modern hybrids along with improvements in production technology (Kiss, 2000).

In the early 1990s the improvement slowed down. Diminishing fertilizer application and consecutive dry years led to a significant decrease in yield.

A great change has recently taken place in maize breeding, the effect of which can be observed worldwide. Besides the demand for high input production on fertile soils, there is an increasing demand for hybrids with multiple stress resistance (e.g. drought, disease and insect tolerance, low N-dose, etc.). These hybrids do not have a clear advantage in favourable or average years, but their superiority becomes obvious in unfavourable years (Kálmán et al., 2003).

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A series of books entitled "Maize Production Technological Trials" edited by Dr. István I'só was published by the Publishing House of the Hungarian Academy of Sciences (Akadémiai Kiadó) between 1953 and 1974, summarizing experiments on production technology in Hungary.

The specific response of maize hybrids to production technology is investigated at the basic research level in three institutions in Hungary:

– Agricultural Research Institute of the Hungarian Academy of Sciences, Martonvásár

– Centre for Agricultural Sciences, University of Debrecen

– Cereal Research Non-Profit Company, Szeged.

Research on agronomy is also carried out by integrating organizations, such as KITE in Nádudvar and IKR in Bábolna.

There is constant debate among maize experts, whether genetic advance or improvements in production technology contribute more to increasing the average yield of maize. Farmers were only able to utilise 37.1–58.0% of the genetic yield potential of maize hybrids in the years 1995–2000 (Kiss, 2001). Consequently, the use of improved production technologies for maize could enable farmers to achieve higher yield averages.

Research has proved that the following factors are essential if the genetic yield potential of maize hybrids is to be manifested: optimum plant density, economical fertiliser rates and timely sowing.

Materials and methods

The meteorological data of 2003 and 2004 are presented in Table 1. The most important traits of new hybrids, bred by conventional breeding, are usually tested for 2–3 years at 5–6 different locations before entering them for official trials. As a result much is known about how the hybrids respond to environmental factors.

The main items in the production technological guide, based on research results, include the use of the hybrid (grain, silage or double-purpose hybrid), the most important traits of the hybrid (e.g. yield potential, yield stability, etc.) and production technology recommendations (e.g. plant density, sowing date, etc.).

Table 1

Meteorological data in the Szeged region in 2003 and 2004

Period and month	Precipitation, mm		Monthly mean temperature, °C		Number of hot and extremely hot days	
	2003	2004	2003	2004	2003	2004
Previous winter half-year	185	248	11.0	12.3	–	–
Vegetation season: April	21	89	20.9	15.8	–	–
May	21	49	24.3	20.3	9	–
June	28	64	24.5	23.4	13	1
July	49	152	24.0	22.8	12	14
August	5	25	–	–	17	5
Total of vegetation season	124	379	–	–	51	20
Total	309	627	–	–	–	–

Production technological trials were carried out on small plots in four replications with optimal soil cultivation. The trials were sown and harvested mechanically, in order to simulate agricultural practice and the most important agronomic properties of the hybrids (plant density, nutrient and planting date responses) were investigated.

Sowing date trials were set up to investigate the effect of sowing date on grain moisture content. In the nutrient response trials the effect of the N rate on the grain yield of the hybrids, the composition of the grain, and the nutrient supplies of the soil was tested. In the spacing trials the effect of plant density was studied on root and stalk lodging, the ratio of barren plants and the quantity of yield.

The size and design of the experimental plots depended on the character of the trial, the demand for reliability, and the possibility of mechanizing the work.

The results were evaluated by variance analysis.

Results

Recently registered Szeged maize hybrids and experimental hybrids expected to be registered shortly were evaluated on the basis of the aspects discussed in the Introduction, using the results of the official Hungarian trials (OMMI) and of trials carried out by breeders (2002–2004). The performance of the hybrids was evaluated over a wide range of ecological environments (yields ranged from 5–13 t/ha) by means of regression analysis. The hybrids were compared either to the official standard/location, or to the average for the hybrids involved in the trial/location (post-registration OMMI trial). The basis of comparison was almost exclusively the better standard variety in the breeders' trials. Only the data of trials with extreme error were excluded from the database.

A total of seven maize hybrids were tested, six of which had above-average drought tolerance. This is a noteworthy result, because 2002, and especially 2003 were extremely dry years (Fig. 1). The performance of the maize hybrid SZE 269 (registered in Slovakia in 2005) provided a convincing argument for the production of early flowering hybrids of the generative type on areas prone to drought. Szegedi SC 352, on the other hand, performs best under intensive conditions. However, as shown in Figure 2, it also gives a consistent yield on average or poorer growing areas.

The data evidence the fact that the breeding strategy has been adjusted to environmental changes in maize production in the past decade. Numerous new hybrids have been introduced, which can tolerate multiple stress effects (drought, lower soil fertility level, viral diseases, etc.) much better. The genetic gain was twice as great under unfavourable conditions than under average ones, averaged over the seven hybrids (Table 2).

The results of production technological trials in 2003 and 2004 can be summarised as follows:

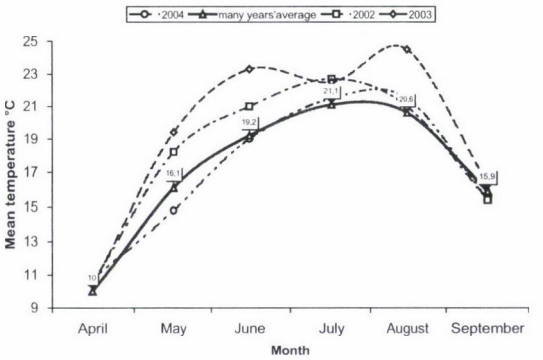

Fig. 1. Mean temperatures in the vegetation seasons of 2002–2004

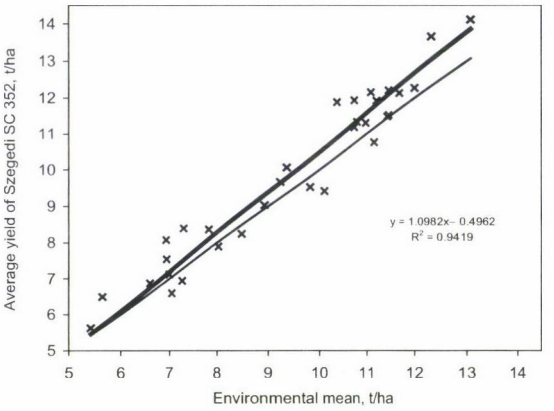

Fig. 2. Results of performance tests on Szegedi SC 352 (in OMMI and GK trials) 2002–2004

Table 2

Comparison of hybrid performance calculated from the trendlines of regression at three different yield levels

Hybrid*	Year of registr.	No. of pairs of data	x–25% (t/ha)	Diff. %	x mean (t/ha)	Diff. %	x+25% (t/ha)	Diff. %	R^2
Szegedi SC 463	2001	30	6.95	*–1.4*	9.27	*–1.7*	11.59	*–1.8*	0.951
Szegedi SC 271	2002	31	6.07	4.9	8.09	4.4	10.11	4.1	0.929
Szegedi SC 352	2002	34	7.07	3.7	9.43	4.6	11.79	5.6	0.942
Szegedi SC 353	2003	17	7.5	2.4	10.02	0	12.53	*–1.4*	0.926
Szegedi SC 470	2005	15	7.49	0.3	9.99	0.1	12.49	*–0.05*	0.948
Szegedi TC 269	2005	14	7.31	10.1	9.74	4	12.18	0.3	0.747
Szegedi SC 433	2006^{+}	7	7.5	4.9	10	0.4	12.5	*–2.3*	0.744

* Database: 1: OMMI + GK 2002–2003; 2: OMMI + GK 2002–2003; 3: OMMI 2002–03, GK 2002–04; 4: OMMI 2003, GK 2002, 2004; 5: OMMI + GK 2002–2003; 6: OMMI 2001–02, GK 2004; 7: OMMI 2003, GK 2004. OMMI = National Institute for Agricultural Quality Control; GK = Cereal Research Non-Profit Company. $^{+}$ Expected date of registration

Response of the hybrids to plant density

Table 3 shows the data of the spacing trials in both years. The data were evaluated with regard to the amount of irrigation water, which was 100 mm in 2003, and 50 mm in 2004. The result of the favourable weather in 2004 was a 24% yield improvement averaged over three hybrids and six plant densities, compared to 2003, when the weather was unfavourable. The impact of the favourable weather was the most striking at a plant density of 70,000 plants/ha (29%).

The data demonstrate the specific plant density responses of the hybrids. In addition, they prove that the highest yield for Szegedi SC 271 and Szegedi SC 352 was recorded at a higher plant density (70 thousand plants/ha) under the more favourable climatic conditions in 2004 and at a lower plant density (60,000 plants/ha) under the unfavourable weather conditions in 2003.

The maize hybrid Szegedi SC 463R, however, performed the best at the same plant density (70 thousand plants/ha) in both years. This hybrid responded with an extra yield of 2.4 t/ha to the more favourable season in 2004.

The plant density responses of the hybrids are tested in trials where the hybrids grow under better conditions than in commercial production. Therefore farmers are recommended to plant the maize hybrids at lower plant densities (10 thousand plants/ha less than in the trials).

N fertilisation of maize

Figure 3 shows the 2003 results of a long-term nitrogen fertilization trial set up in 1979. The highest grain and starch yields were obtained with 210 kg/ha nitrogen active agents, the highest protein yield with 280 kg/ha, while the economic optimum was 140 kg/ha nitrogen in all cases.

Table 3
Plant density (thousand plants/ha) response of hybrids in 2003 and 2004

Plant density	Grain yield t/ha											
	Szegedi SC 271			Szegedi SC 352			Szegedi SC 463 R			Average		
	2003	2004	%*	2003	2004	%	2003	2004	%	2003	2004	%
40	7.1	8.4	118	7.5	10.3	137	7.9	8.3	105	7.5	9.0	120
50	7.8	9.5	122	8.3	10.5	126	8.2	9.3	113	8.1	9.8	121
60	8.8	10.2	116	8.8	12.0	136	9.0	10.8	120	8.9	11.0	124
70	8.8	10.9	124	8.9	12.3	138	9.6	12.0	125	9.1	11.7	129
80	9.0	11.3	114	8.7	12.1	139	9.5	11.1	117	9.1	11.5	126
90	9.0	11.1	112	8.8	11.7	133	9.4	10.8	115	9.1	11.2	123
Mean	8.4	10.2	121	8.5	11.5	135	8.9	10.4	116	8.6	10.7	124
$LSD_{5\%}$	0.4	0.7	–	0.4	0.4	–	0.6	0.8	–	–	–	–

* 2003 data = 100%

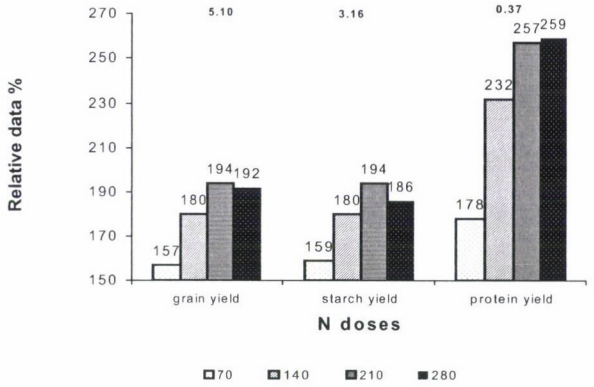

Fig. 3. Effect of nitrogen fertilisation on the grain yield, starch yield and protein yield of maize averaged over 12 hybrids. Újszeged, 2003. Value of the check = 100%

The difference in the nitrogen response of the cultivars is demonstrated by a comparison of two hybrids (Table 4). Szegedi SC 352 yielded 36% more on the untreated check plot than Ella. Both hybrids had nearly the same yield on the treated plots. The yield-increasing effect of fertilisation was considerably higher for Ella than for Szegedi SC 352.

Szegedi SC 352 proved to be better because its yield on the fertilised plots was nearly the same as that of Ella, while it utilised the natural nutrient supply of the soil on the check plot significantly better than Ella.

Response of hybrids to sowing date

The results are shown in Table 5. The soil was too wet in 2004 for sowing to be carried out at the earliest date. Under the dry weather conditions in 2003 late sowing diminished the grain moisture content by only 1% compared to the earliest sowing date but resulted in a significant yield decrease (7%). Under wet conditions in 2004 late sowing increased the grain moisture content at harvest by 2.6% compared to sowing on 22nd April, but the reduction in yield was not significant (4%). The highest yield (10.2 t/ha), associated with 1.3% higher grain moisture content, was recorded after sowing on 3rd May. Grain moisture increased by an additional 1.3%, and yield decreased significantly (7%) when sowing was delayed until 11th May.

The specific sowing date responses of the hybrids can be summarized as follows:

2003: Sowing on 7th May increased the grain moisture content of Szegedi SC 352 at harvest the least (0.2%), and that of Szegedi TC 367 the most (2.0%) compared to sowing on 11th April; it diminished the yield of Szegedi SC 271 least (0.0%) and that of Szegedi TC 367 and Szegedi TC 377 the most (0.6 and 0.9 t/ha).

Table 4
Nitrogen response of different hybrids averaged over trial results in 2003–2004
Újszeged, 2003–2004

Fertilizer active agent rate (kg/ha)			Ella			Szegedi SC 352			%*
N	P_2O_5	K_2O	t/ha	D	%	t/ha	D	%	
0	0	0	5.0	–5.3	100	6.8	–2.8	100	136
70	150	150	8.5	–1.8	170	8.6	–1.0	126	101
140	150	150	10.3	0.0	206	9.6	0.0	141	93
210	170	200	10.4	0.1	208	10.8	1.2	159	104
280	170	200	10.3	0.0	206	10.1	0.5	149	98
Average of fertilised plots			9.9	–	198	9.8	–	144	–
$LSD_{5\%}$			1.1			1.0		–	–

*Yield of Ella = 100%

Table 5
Effect of planting date on the grain moisture content and yield of ten hybrids
Újszeged, 2003–2004

Year		Grain moisture content at harvest		Grain yield	
	Sowing date	%	D	t/ha	%
2003	Apr. 11	19.0	0.0	8.9	100
	Apr. 18	19.2	0.2	8.9	100
	Apr. 28	19.2	0.2	8.7	98
	May 7	20.0	1.0	8.3	93
	$LSD_{5\%}$	–	–	0.4	5
2004	Apr. 22	19.6	0.0	9.9	100
	May 3	20.9	1.3	10.2	103
	May 11	22.2	2.6	9.5	96
	$LSD_{5\%}$	–	–	0.5	5

2004: Sowing on 11th May increased the grain moisture content of Szegedi SC 463R at harvest the least (1.5%), and that of Szegedi TC 367 the most (3.2%) compared to sowing on 22nd April. It diminished the yield of Szegedi SC 271 the most (1.7 t/ha), and that of Szegedi 269 the least (0.1 t/ha).

Discussion

Four maize hybrids from Szeged were registered in Hungary during the period of this project and the registration of five further Szeged hybrids is expected in 2005–2006. The yield potential and yield stability of the hybrids are tested for 3–4 years at nearly 100 locations in small-plot and large-scale trials, in cooperation with other research institutions, integrators (e.g. KITE) and farmers.

In order to exploit the yield potential of the maize hybrids to the highest possible extent in commercial maize production, farmers are provided with a production technology specific to the hybrids.

The production technological recommendations are based on the knowledge of the specific properties of the hybrids, based on the results of agronomic trials.

Within the framework of the project the specific responses of the hybrids to plant density, nitrogen fertilizer and sowing date were tested. It was concluded that hybrids with different vegetation periods have significantly different agronomic traits.

1. The plant density optimum of the hybrids per hectare is given as a plant density range, because the response of the hybrids to plant density may vary depending on location, weather, and the standard of the production technology.

2. The planning of the nitrogen rate/hectare is based on the principle that the nutrients extracted from the soil by crop production must be replaced. The various responses of the hybrids to nitrogen must be considered when planning the yield and the nitrogen rate. The best hybrids for commercial production are those which can utilise the naturally available nutrient supply of the soil, and respond to an increase in the nitrogen rate with an average or higher yield increase.

3. Sowing later than the optimum date increases the grain moisture content at harvest to a different extent for each genotype, and reduces the quantity of grain yield.

Acknowledgements

Thanks are due to the Ministry of Education for grant No. 4/0008/2002 (National Research and Development Programme; sub-programme Research in Agrarian Economy and Biotechnology); to the Centre of Agricultural Sciences, University of Debrecen, for heading the consortium, for organizing the project, and allowing us to participate; to the management of the Cereal Research Non-Profit Company and to colleagues who contributed to the project, and to Mrs. L. Buza for her administrative assistance.

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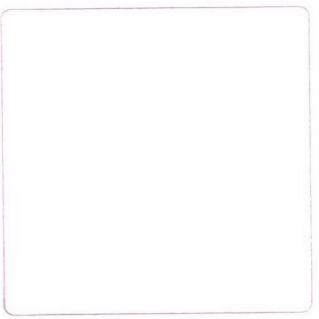

Acta Agronomica Hungarica, 53(2), pp. 153–159 (2005)

WATER LOSS DYNAMICS IN MAIZE HYBRIDS WITH DIFFERENT GENOTYPES

A. C. DOBOS and G. SZABÓ*

DEPARTMENT OF LAND USE AND REGIONAL DEVELOPMENT, HAS-DU
CENTER FOR AGRICULTURAL SCIENCES, DEBRECEN UNIVERSITY, DEBRECEN, HUNGARY
* KITE AGRICULTURAL SERVICE AND COMMERCIAL PLC., NÁDUDVAR, HUNGARY

Received: 29 March, 2005; accepted: 27 May, 2005

Tests were made on the water dynamics of water loss in maize at two locations at major maize production areas, Debrecen and Szeged, as a function of the heat sum required up to physiological ripening.

The maximum water content was found to vary according to the hybrid and the year. No significant differences were found between the effective heat sums associated with maximum values within a year, the difference being 2–4 calendar days. There was a close correlation between the maximum grain water content and the length of the generative phase for a given hybrid in a given year. Better water supplies (1998) resulted in higher maximum water content values, which indirectly promoted longer and more intensive dry matter incorporation.

Of all the environmental parameters, the drying down rate is primarily determined by the temperature. The changes in grain moisture content in a specific hybrid can be characterised by a joint linear graph. The drying down rate of the individual hybrids was determined by examining the b term of the linear equation.

Key words: drying down, black layer, heat sum

Introduction

Water is a natural component of maize grain, and has a significant effect on physiological processes, grain quality and profitability. The optimal maize grain water content for combine harvesting is 23–24% but a much lower water content is favourable for storage (Bocz, 1992).

The formation of grain water content can be divided into two important periods: active physiological processes exert their effects during grain filling, and passive dehydration processes after physiological ripening (Schmidt and Hallauer, 1966). The correlation between temperature and the grain water content is significant in the 75–50% and 50–30% range, but it is not the only influencing factor in the second period (Fótos, 1983). Besides temperature, other weather factors (relative moisture content, saturation deficiency) also have a significant role in the following period (at a water content of below 30%). According to Aldrich and Leng (1972), weather has a greater influence than other factors following physiological ripening. During grain filling, plant cells go through cycles of division, elongation and dormancy. All have an effect on grain water content, which first increases in mass and then decreases. Grain moisture content is also an important indicator of maize grain ripening (Hallauer

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and Russell, 1962). A positive direct effect has been detected (Kang et al., 1986) between grain filling and drying down (in terms of Growing Degree Days). The dynamics and length of drying down depend greatly on climatic factors. According to Bloc and Gouet (1974) temperature is the most important influencing factor. A negative correlation has been detected (Kang et al., 1986) between grain weight and ear water content (–0.84), but after physiological ripening, the role of grain weight is insignificant (r=0.04).

When examining the drying down period (from black layer formation to harvest), Schmidt and Hallauer (1966) found that the daily grain moisture decrease depended on the year. Significant hybrid specificity was not observed by Hicks et al. (1976) in studies on the dynamics of drying down. In contrast, Purdy and Crane (1967), Sutton and Stucker (1974) and Hallauer and Russell (1962) detected significant differences between certain lines and hybrids.

It can be said that the optimal level of most cultivation technology measures (plant density, fertilization) does not only increase yield, but also decreases the grain moisture content (Kising, 1962; Nagy and Zeke, 1981). In maize, satisfactory nutrient supplies do not only increase yield, but also improve the water utilization of the plants (Kovács, 1982; Nagy, 1996; 1998; Németh and Búzás, 1991). Irrigation significantly increased the grain moisture content of many hybrids, especially in the case of low nutrient supplies (Nagy and Zeke, 1982).

Materials and methods

Samples were taken in two locations, Debrecen and Szeged, which are major maize-growing areas.

Debrecen

The three-factor (fertilization, irrigation, genotype) experiment was set up by the Centre of Agricultural Sciences, Debrecen University in collaboration with KITE PLC., Nádudvar, at the Látókép Experimental Station of the university in 1978. The productivity, natural nutrient utilization ability and fertilizer response of 10 maize hybrids were examined each year. The experiment was laid out in a split-plot design with four repetitions with fertilization treatments in the main plot (120 m^2) and maize hybrids in the subplots (15 m^2). Plots of similar size were used for destructive samplings.

The Experimental Station is situated on the Hajdúság Loess Ridge, and the soil is a lowland chernozem with lime deposits and a deep humus layer, formed on loess. It has medium hard loam texture. The groundwater is located at a depth of 5–8 m (Rátonyi, 1999).

Szeged

The ripening biology of maize hybrids was studied in an experiment set up by KITE PLC, Nádudvar, in collaboration with the Cereal Research Institute (CRI), Szeged. Each year, 12–20 of the top commercially grown hybrids were examined in three replications with 50 plants in a plot, with 70 × 24 cm spacing and a plant density of 59,500 plants per hectare. The soil type is a solonetz, strongly calcareous, meadow chernozem, with loam or sandy loam texture. The soil has good water permeability and satisfactory water retention, but has a tendency to become excessively wet in the case of high water levels.

The daily precipitation sum was determined by local measurements, while the daily radiation and temperature data were provided by the Agrometeorological Observatory Centre of Agricultural Sciences, Debrecen University, and by the National Meteorological Service in Budapest. Among the agrometeorological parameters, an analysis was made of the precipitation during the growing season, effective heat sums during the vegetative and generative phase, and the water supplies. The daily heat sums were determined using the algorithm proposed by Ritchie et al. (1994).

In 1997, the total rainfall was 394.7 mm in Debrecen, which is 188.3 mm less than the average over many years. Of this, 113 mm fell from the harvest of the forecrop to sowing. In 1998 there was 157 mm of precipitation during the winter half-year, which was 86 mm less than the average over many years. However, during the growing season (from the first ten days of May to the first ten days of October) there was 470.9 mm of precipitation, exceeding the 50-year average by 130.9.

Like 1998, 1999 was wetter than average, with a total rainfall of 635.4 mm, including 389.2 mm during the growing season.

In Szeged, there was only 282 mm of precipitation during the growing season, but due to the favourable water supplies during the critical periods, the development and growth of maize was unaffected. The cold wet May not only delayed sowing but also delayed the emergence of the seedlings. Due to the favourable climatic conditions in July, the plants were able to develop at an optimal rate, so flowering was only delayed by 2–3 days.

The years 1998 and 1999 were both favourable. Rainfall amounted to 382 mm in 1998 and 463 mm in 1999. During germination and flowering, the rainfall supplies were adequate, so the early development of the plants was close to optimal. The temperature was moderate, as expected in wetter years, so there were no lengthy hot spells.

In Debrecen, destructive sampling was carried out after flowering in 1998, weekly in 1999 and every 3 days on average in 1997.

In the course of sampling the weight of 50 grains from the middle section of 4 ears was measured in 4 replications in Debrecen. Dry matter content was determined after drying to constant weight in a drying cabinet at 60°C.

In Szeged, samples were taken once a week from the end of August. Each sample consisted of 3 ears per replication and the grain was dried to constant weight, while gradually increasing the temperature to 105°C.

The drying down dynamics of the hybrids was evaluated at both growing sites in the 90 kg N/ha fertilizer treatments, using a generalised linear model (GLM) and regression analysis. The statistical analysis of the GLM model was complemented with linear regression analysis. In order to determine the goodness of fit, multiple R values, the standard error of estimation and the confidence intervals of certain subsets were examined (Huzsvai, 2000). The statistical evaluations were carried out using SPSS for Windows 9.0 and Excel XP software.

Results

Water is a natural constituent of maize grain, and has a significant effect on physiological processes, grain quality and profitability. The optimum grain water content is 23–24% for combine harvesting, but a much lower water content is favourable for storage. After flowering, most physiological processes are oriented towards the ear, to facilitate dry matter incorporation, which is accompanied by changes in the grain moisture content.

The drying down dynamics of the grain was evaluated at two locations, Debrecen and Szeged, in the 90 kg N/ha fertilizer treatment, as a function of the heat sum until the formation of the black layer.

On the basis of the results, as also reported in the literature, the generative phase could be divided into three phases from the point of view of drying down (Fig. 1).

Phase of maximum water weight

The phase lasting until maximum water weight was examined using the 1997 and 1998 data of the Debrecen location. The most accurate approximation was achieved with a quadratic function for both hybrids (Figs. 2 and 3). In addition to the multiple R value, the variance analysis of regression, the F-probe and the standard error of estimation were used to determine the goodness of fit. The multiple R-squares were high, and the standard errors were located in the 8.15–24.5 g range.

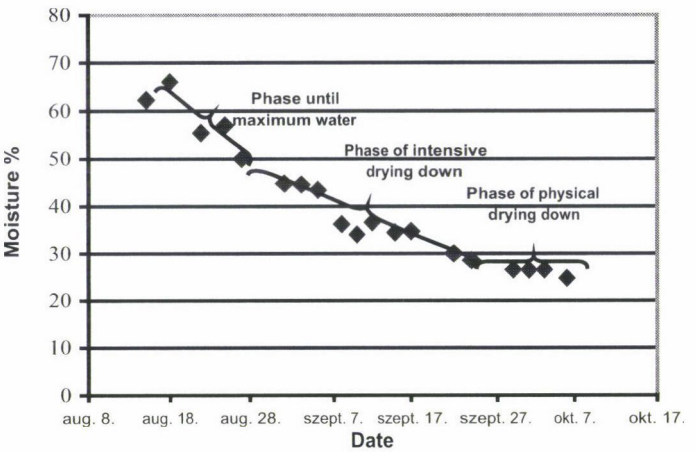

Fig. 1. Phases of drying down (Debrecen, 1997)

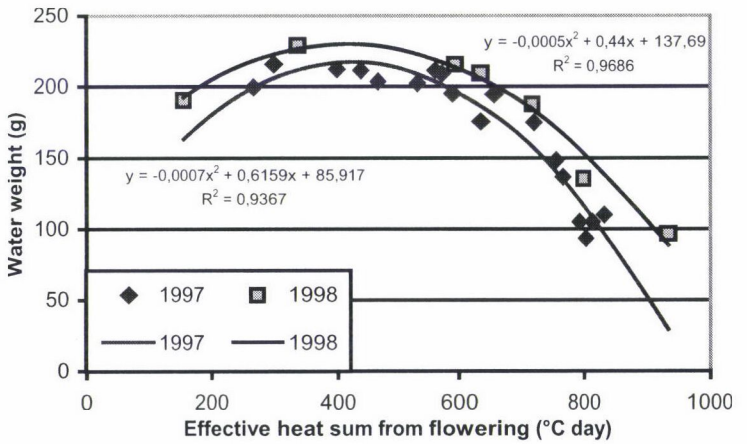

Fig. 2. Changes in grain water weight during the generative phase (MV 484 SC) (Debrecen, 1997–1998)

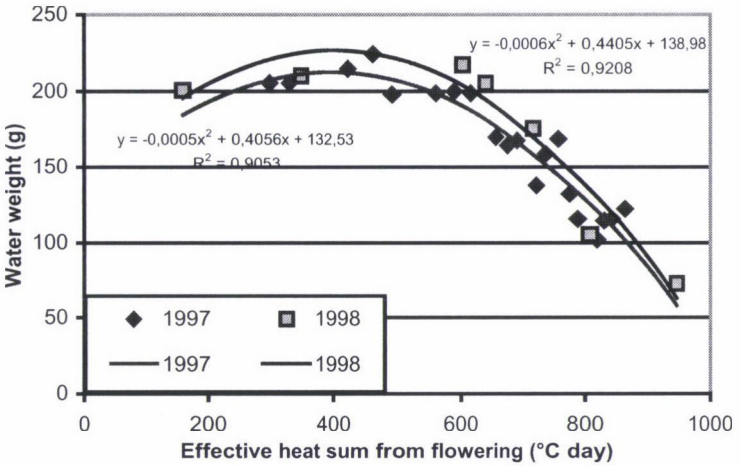

Fig. 3. Changes in grain water weight during the generative phase (DK 471 SC) (Debrecen, 1997–1998)

The maximum water weight for each hybrid was calculated along with the effective heat sum (Table 1). The maximum water weight in 1997 was 215 g for the hybrid DK 471 and 221 g for MV 484. The average heat sum associated with maximum water weight was 423 degree days. The hybrids differed from this value by ± 17 degree days, which is equivalent to about 2 calendar days. The maximum water weight in 1998 was 220 or 235 g, the higher value again being recorded for MV 484 SC. The average heat sum associated with maximum water weight was 403 degree days. The hybrids differed from this value by ± 37 degree days, equivalent to about four calendar days.

When comparing the two years, it can be seen that the increase in grain water weight lasted 20 degree days longer on average in 1997, but had lower intensity. The higher water weight values recorded in 1998 were associated with a longer generative phase.

The intensive phase of drying down

The moisture % data recorded in Debrecen and Szeged were used to examine the intensive phase of drying down. The data covered the period from the end of August to the formation of the black layer.

The first part of the evaluation aimed to determine whether there were any significant differences between the years for each hybrid and location (Tables 2–3). It was found that the dynamics of drying down for a specific genotype could be approximated by a common regression line and the years did not differ significantly from each other. Linear fitting (multiple r-squares) was close in all cases.

Table 1
Relationship between maximum water weight and the length of the generative phase of hybrids (Debrecen, 1997–1998)

Hybrid	1997			1998		
	X_{max}	Y_{max}	Length of the generative phase (degree days)	X_{max}	Y_{max}	Length of the generative phase (degree days)
DK 471 SC	406	215	818	367	220	807
MV 484 SC	440	221	792	440	235	799

Table 2
Drying down dynamics of maize hybrids with different genotypes in the intensive phase (Szeged, 1997–1999)

Hybrid	FAO No.	Years			
		1997	1998	1999	Common regr. equation
DK 471	410	–0.0827x+98.514 R^2 =0.998	–0.0676x+86.802 R^2 =0.987		–0.072x+90.296 R^2 = 0.97876
MV 484	450	–0.0702x+89.099 R^2 = 0.961	–0.0752x+80.980 R^2 = 0.967	–0.0717x+89.48 R^2 = 0.997	–0.0735x+84.149 R^2 = 0.987

Table 3
Drying down dynamics of maize hybrids with different genotypes in the intensive phase (Debrecen, 1997–1999)

Hybrid	FAO No.	Years			
		1997	1998	1999	Common regr. equation
DK 471	410	–0.0843x+93.576 R^2 = 0.946	–0.0912x+99.487 R^2 = 0.984	–0.077x+87.459 R^2 =0.999	–0.076x+90.711 R^2 = 0.955
MV 484	450	–0.0686x+83.362 R^2 = 0.730	–0.0795x+90.095 R^2 = 0.984	–0.066x+80.032 R^2 = 0.996	–0.0715x+84.805 R^2 = 0.887

The next step examined how the location influenced the dynamics of drying down in the hybrids MV 484 SC and DK 471 SC. The rate of drying down was similar in Debrecen and Szeged, which both had good soil conditions for maize production. The change in moisture content at these two locations can be described with a common regression coefficient as a function of the effective heat sum (MV 484 SC: b_b= – 0.0723; DK 471 SC: b_b= –0.0718).

However, a common linear regression equation cannot be applied to the two locations, since the constants differ significantly from each other. It can be assumed that, at certain locations, other factors besides the temperature (soil characteristics, rainfall distribution) also influence the drying down dynamics to some extent. The b term of the linear function was analysed to determine the drying down rate of each hybrid at each location.

Acknowledgements

This research was funded by the NKFP maize consortium (No. 4/008/2002).

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Acta Agronomica Hungarica, 53(2), pp. 161–175 (2005)

EVALUATION OF THE SOIL WATER REGIME OF AN IRRIGATED MAIZE FIELD

C. FARKAS, A. HAGYÓ, E. TÓTH, J. SZABÓ and T. NÉMETH

RESEARCH INSTITUTE FOR SOIL SCIENCE AND AGRICULTURAL CHEMISTRY
OF THE HUNGARIAN ACADEMY OF SCIENCES, BUDAPEST, HUNGARY

Received: 17 March, 2005; accepted: 31 May, 2005

This study was carried out to evaluate the soil hydrophysical properties and soil water regime of two irrigated maize fields in order to support irrigation planning and management. The experimental sites were located in Mezőhegyes (MZH) and Hódmezővásárhely (HMV) in SE Hungary. In total 11 monitoring stations were chosen, using information from a previously developed, GIS-based agro-geoinformation system. In 2003 and 2004 soil sampling and *in situ* measurements were performed to determine the soil hydrophysical properties and soil water content dynamics. The hydraulic conductivity of the topsoil was evaluated from double ring infiltrometer measurements. A previously calibrated TDR 300 instrument and a 3T-M capacitance probe were used for quantifying the soil water content. Both types of equipment were found to require calibration and testing under field conditions before use. It was concluded that the study fields could be considered relatively homogeneous in relation to both soil hydrophysical properties and soil water regime. Thus, monitoring stations established for one or two carefully selected soil profiles could provide enough data to ensure proper decisions on irrigation. The results indicate that the soil management system and irrigation strategy used in the experimental fields ensured satisfactory soil and soil moisture conditions.

Key words: maize, soil water, double ring infiltrometer, TDR 300, capacitance probe

Introduction

Shortage of water resources has become a major concern for sustainable crop production (Zhang et al., 2004) in many parts of the world. In Hungary water deficit during the vegetation period is fairly common. The limited water resources are becoming more and more expensive and are likely to become the main restraints to agricultural development and nature conservation (Várallyay, 1997). Hence, the evaluation of new cropping, tillage and irrigation methods to increase soil water-holding capacity and crop water use efficiency is of great importance.

Viewed from the perspective of water stress, the purpose of irrigation is to keep the water status at a level that maximises yield within the constraints of irrigation supply and growing season weather (Aujla et al., 2005). Several efforts have been made to use irrigation water as efficiently as possible under different cultivation systems. Based on research findings, Harmanto et al. (2005) noted that crop water requirements might differ from one region to the other. Consequently, it is important to analyse the soil water regime of irrigated agricultural fields under different climate and soil conditions. On the other hand, it is essential to give practical advice to farmers to promote the sustainable and economical agricultural utilization of soils.

0238–0161/$ 20.00

Maize is one of the main crops grown in Hungary. Yields, however, greatly depend on the amount of precipitation in the given year, because under Hungarian conditions the potential evapotranspiration of maize stands exceeds the precipitation sum of the same period even in relatively wet years (Antal, 1966; Szász, 1988).

At the Research Institute for Soil Science and Agricultural Chemistry of the Hungarian Academy of Sciences, a GIS-based agro-geoinformation system was developed for the Mezőhegyes and Hódmezővásárhely experimental sites for use in analysing the relationship between site-specific characteristics and the crop yield, and for making decisions on the optimisation of cultivation systems and soil protection (Szabó et al., 2004). This system is based on the GIS adaptation and digital reambulation of large-scale soil information originating from various agro-geological surveys (Pásztor et al., 2002). Within the new system, the soil water regime is of special interest. Thus, numerous factors determining the soil water regime and the crop water capacity must be taken into consideration in the planning and operating of irrigation conforming to the requirements of both soil and crop.

The objectives of the present work were to analyse the soil physical properties and soil water regime of two irrigated fields, making use of the previously developed agro-geoinformation system, and to evaluate affordable and easily usable techniques for measuring soil water content and infiltration that could be recommended to farmers for making decisions on irrigation.

Materials and methods

Description of the study area

The experimental sites are located near Mezőhegyes (MZH) and Hódmezővásárhely (HMV) on the Csongrád Plain in SE Hungary. This plain is situated between 80 and 101 m above sea level and slopes towards the Tisza valley. The plain is not prone to flooding and there are many depressions in its surface. The clay and silt sediments are covered with a loess layer, which becomes thicker from East to West. The climate of the Csongrád Plain is warm and dry, and moderately warm on the North part. The number of sunshine hours ranges from 2000 to 2050, and the annual mean temperature from 10.2 to 10.6°C. The precipitation values vary between 550 and 600 mm, with 300 to 350 mm rainfall during the growing season. The soil cover of the Csongrád Plain is very diverse, but chernozem soils cover 80% of the area.

On both the experimental sites, various maize hybrids were grown, using a modern automatic linear irrigation technology. The amount of rainfall and irrigation water in 2003 is shown in Figure 1.

Based on the available information on elevation, soil types, and soil physical and chemical properties (Pásztor et al., 2002; Szabó et al., 2004), four reference soil profiles were chosen in each of the study fields to represent the range of variation in soil properties within the examined fields.

In 2003, monitoring stations (MZH1–MZH4 and HMV1–HMV4) were established next to the representative profiles. Figure 2 demonstrates the spatial patterns of the soil physical properties according to the available soil maps and the location of the monitoring stations at the Mezőhegyes experimental site.

In 2004, a new experimental field with three reference soil profiles (MZH5–MZH7) was chosen for further investigations.

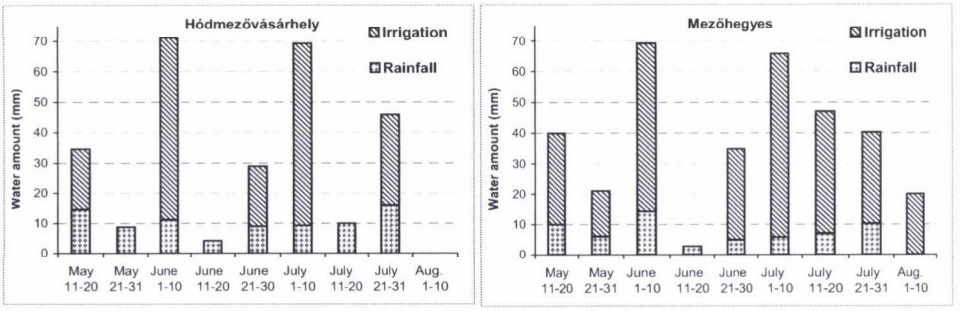

Fig. 1. Amount of rainfall and irrigated water during the 2003 vegetation period on fields located near Hódmezővásárhely (left) and Mezőhegyes (right)

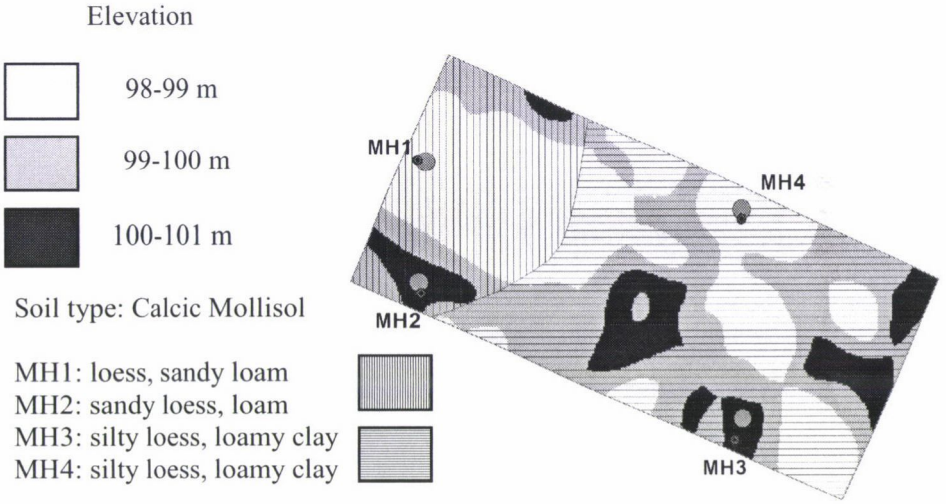

Fig. 2. Description of the Mezőhegyes experimental site

Soil sampling and analyses

In the first year of the study, disturbed and undisturbed (100 cm^3) soil samples were taken from three soil horizons in each of the eight soil profiles (MZH1–MZH4 and HMV1–HMV4). The disturbed soil samples were used to determine the particle size distribution using the pipette method (Buzás, 1993). The undisturbed soil cores were used for the evaluation of bulk density (bd) and soil water retention values. The latter were measured at pressure heads represented by pF values of 0.0, 0.4, 1.0, 1.5, 2.0, 2.3, 2.7, 3.4 and 4.2 according to Várallyay (1973). The soil water retention data were expressed in terms of volumetric water content using the bulk density of the individual cores. The potential amount of plant-available water (PAW) was calculated as the difference between the field capacity ($\Theta_{pF=2..3}$) and wilting point ($\Theta_{pF=4.2}$) values.

In 2004 undisturbed soil samples were taken from three soil layers (5–10, 25–30 and 45–50 cm) in five replicates at the MZH5–MZH7 locations. Three replicates were used to measure the saturated hydraulic conductivity, using the hanging water column approach, while the soil water retention characteristics were determined from the other two soil samples.

In situ *soil hydraulic conductivity measurements*

In June 2004, *in situ* double ring infiltrometer measurements were performed at two locations (MZH5 and MZH6) to evaluate the saturated hydraulic conductivity of the topsoil (Fig. 3) (Daniel, 1989). The rings were installed in the soil and filled with water. The water level in the larger ring was kept constant during the measurements to ensure the vertical infiltration of water from the smaller ring. Changes in the soil water level were detected in the smaller ring as a function of time. Two parallel measurements were carried out at each location.

Regression curves were fitted to the infiltration rates (IR) calculated from the measured data, using the following logarithmic expression: $IR = A*\ln(t) + B$, where t is the time and A and B are fitted parameters. Saturated hydraulic conductivity values were estimated from the convergence of the infiltration rate curves.

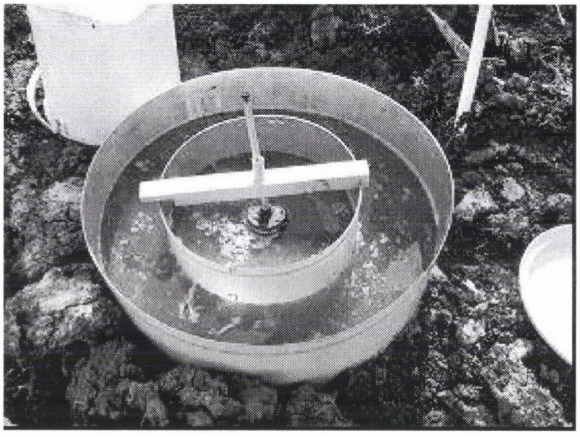

Fig. 3. Double ring infiltrometer used to measure soil hydraulic conductivity

Soil water content measurements

Volumetric soil water content (Θ) and soil temperature measurements were carried out in the reference soil profiles every 10 cm up to a depth of 80 cm on seven occasions during the 2003 vegetation period, using a 3T-M type capacitance probe (Szőllősi, 2003). At the same time, TDR 300 equipment (Topp and Davis, 1985; Rajkai, 2004) with 20 cm electrodes was used to determine the volumetric soil water content of the upper 0–20 cm layer. Previously, both the instruments were calibrated on soil samples taken from the reference soil profiles.

In 2004, disturbed soil samples were taken at the MZH5–MZH7 locations from the 5–10, 25–30 and 45–50 cm soil layers to evaluate the soil water content dynamics.

Using the measured soil water content data, the total amount of water stored in the soil profile up to a particular (20, 30, 50 and 80 cm) depth was calculated for different dates, according to Equation 1:

$$TSW_z = \sum_{i}^{n} \Theta_i * T_i \; ; \qquad \text{(Eq. 1)}$$

where: TSW_z – total amount of water present in the soil at a depth of z (mm)

T_i – thickness (mm) of soil layer i

$z = \sum_{i=1}^{n} T_i$

Θ_i – volumetric water content (m^3/m^3) of soil layer i

n – number of layers in the soil profile.

Calibration of the TDR 300 instrument

The calibration of the TDR 300 probes was performed on soil samples taken from the experimental sites and inserted into plastic cylinders 0.4 m high and 0.1 m in diameter. The bulk density of the soil was adjusted to 1500 kg/m^3. The necessary amount of water was then added to each cylinder to ensure the following reference soil water content values for the calibration: 22, 25, 30, 36, 41 and 46 v% for soil samples taken in Hódmezővásárhely, and 31, 36, 39, 44 and 49 v% for soil samples taken in Mezőhegyes. In each cylinder, soil water content measurements were performed in three replicates, using the TDR 300 instrument with 20 cm electrodes. Soil samples were then taken from the cylinders and the soil water contents were verified using the oven-drying method. The measured soil water content values were then compared with the reference ones.

Calibration of the 3T-M capacitance probe

The calibration of the 3T-M capacitance probes was performed under both laboratory and field conditions.

Because of the length of the instrument (80 cm, consisting of 8 capacitance probes, each 10 cm long) and difficulties in ensuring uniform water distribution within a long soil column, only the 6th, 7th and 8th probes, located at the end of the tube, were calibrated in the laboratory. A container 34 cm in height and 27.5 cm in diameter was used for this purpose. Soil previously taken from one chosen profile on each of the study sites was inserted into the container, to give a bulk density of 1500 kg m^{-3} and a soil water content of about 34 v%. Meanwhile, the measurement tube of the 3T-M instrument was placed in the soil. Following soil water content measurements in the 0–10, 10–20 and 20–30 cm layers, undisturbed soil samples were taken from the container from three depths in three replicates to determine the volumetric soil water contents obtained at the 0–5, 5–10 and 10–15 cm depths. The 3T-M measurements were transformed into Θ values and compared with the soil water contents measured in the undisturbed soil samples.

Calibration at the experimental site consisted of performing soil water content measurements with the 3T-M probe every 10 cm up to a depth of 50 cm at three locations and taking undisturbed soil samples from the relevant soil layers (3–8, 13–18, 23–28, 33–38 and 43–48 cm) at the same time. The soil bulk density and soil water content were determined from the soil samples. These values were used as reference data.

Results

Precipitation and irrigation

The precipitation sums (P_{2003}) during May, June and July 2003 were far below those recorded in years with high maize yields at both the experimental sites, and were also much smaller than the potential evapotranspiration (PET) of maize stands (Table 1). Consequently, irrigation (I) was crucial during this period. The total amount of water (P+I) received by the maize stand was in good agreement with the PET values, indicating that no water stress occurred. These values, however, are given on a monthly basis and do not provide information on the daily dynamics of the soil water content.

Evaluation of soil water retention curves

The main physical and hydrophysical properties of different layers in the representative soil profiles are given in Table 2. Water contents at saturation varied from 48–51 v% and 46–47 v% for the 10–15 cm layer and from 43–46 v% and 47–51 v% for the 30–35 cm layer in MZH and HMV, respectively. No

differences exceeding the measurement error (5%) were found in the field capacity values of soils taken from different monitoring stations in the same field. The differences observed between the potential amounts of plant-available water within the study field were small.

Table 1

Potential evapotranspiration (PET, mm) characteristic of maize fields, along with the precipitation and irrigation values (mm) measured on the experimental fields in 2003

		May	June	July	Total
MZH	P_m	67	80	64	211
	PET_{wet}	45	102	127	274
	PET_{dry}	54	98	194	346
	P_{2003}	16	22	23	61
	$P_{2003} + I_{2003}$	61	137	153	351
HMV	P_{2003}	23	24	35	82
	$P_{2003} + I_{2003}$	43	104	125	272

P_m: mean precipitation sum (mm) of years with high yield, source: Szász, 1988; PET: potential evapotranspiration values (mm) measured on maize fields in relatively dry and wet years; source: Antal, 1966; P: precipitation (mm); I: irrigation (mm)

Table 2

Physical properties of different layers of the soil profiles

Site	Soil layer (cm)	Particle size distribution (%)			Bd (g/cm^3)	$\Theta_{pF=0}$ (v%)	$\Theta_{pF=2.3}$ (v%)	$\Theta_{pF=4.2}$ (v%)	PAW (v%)
		Sand	Silt	Clay					
MZH1	10–15	2.1	66.7	31.2	1.28	50.7	31.8	16.8	17.5
	30–35	6.4	62.7	30.9	1.47	46.3	38.7	19.8	16.1
MZH2	10–15	6.1	64.0	29.9	1.39	47.7	35.6	17.8	17.1
	30–35	7.7	61.7	30.6	1.48	45.1	37.3	19.9	16.1
MZH3	10–15	3.7	66.9	29.4	1.36	48.2	35.0	17.8	16.7
	30–35	7.0	62.7	30.3	1.52	43.5	37.7	21.0	16.3
MZH4	10–15	5.4	61.7	32.9	1.27	50.6	33.9	18.0	16.7
	30–35	5.3	59.4	35.3	1.42	46.0	39.4	19.5	15.3
HMV1	10–15	9.9	56.6	33.5	1.49	46.1	37.3	19.8	15.0
	30–35	8.2	57.3	34.5	1.37	50.6	35.2	19.1	18.9
HMV2	10–15	13.4	56.3	30.3	1.41	46.5	36.1	19.0	17.7
	30–35	13.1	56.0	30.9	1.46	47.1	36.5	20.4	17.5
HMV3	10–15	17.4	56.3	26.3	1.45	46.8	36.3	19.6	17.2
	30–35	11.2	61.4	27.4	1.46	47.0	36.8	20.4	16.7
HMV4	10–15	12.2	56.2	31.6	1.46	46.7	36.7	20.0	15.9
	30–35	15.0	55.7	29.3	1.43	49.2	35.8	20.5	19.8

bd: bulk density (g/cm^3); $\Theta_{pF=0}$: water content at saturation (v%); $\Theta_{pF=2.3}$: field capacity (v%); $\Theta_{pF=4.2}$: wilting point (v%); PAW: potential amount of plant-available water (v%)

The shapes of the soil water retention curves determined for the Mezőhegyes field (Fig. 4) differed in the low suction range, indicating that the differences were mainly related to the structural status of the soil, which can easily be influenced by mechanical disturbance (tillage operations, trampling, biological activity, etc.). Since the shape of the pF-curve in the low suction range (pF<2-) does not have a direct effect on the amount of plant-available water stored in the soil, it was assumed that the variation in pF-curves did not cause significant differences in the soil water contents of the different locations.

Double ring infiltrometer measurements

The infiltration curves measured using double ring infiltrometers are shown in Figures 5 and 6. The infiltration capacities were approximately 288 and 648 cm/day for the MZH5 and MZH6 sites, respectively. These values are about one order of magnitude larger than those measured in the laboratory with the constant head method (Table 3). The 100 cm^3 undisturbed soil cores used for the laboratory measurements do not represent the inter-aggregate porosity. Consequently, no water flow through the biopores (root and earthworm channels, etc.) can be considered. Moreover, these measurements are strongly influenced effect exerted by the side of the cylinder. It can be supposed that *in situ* measurements performed on a relatively large soil surface gave better estimates of the saturated hydraulic conductivity compared to the laboratory measurements.

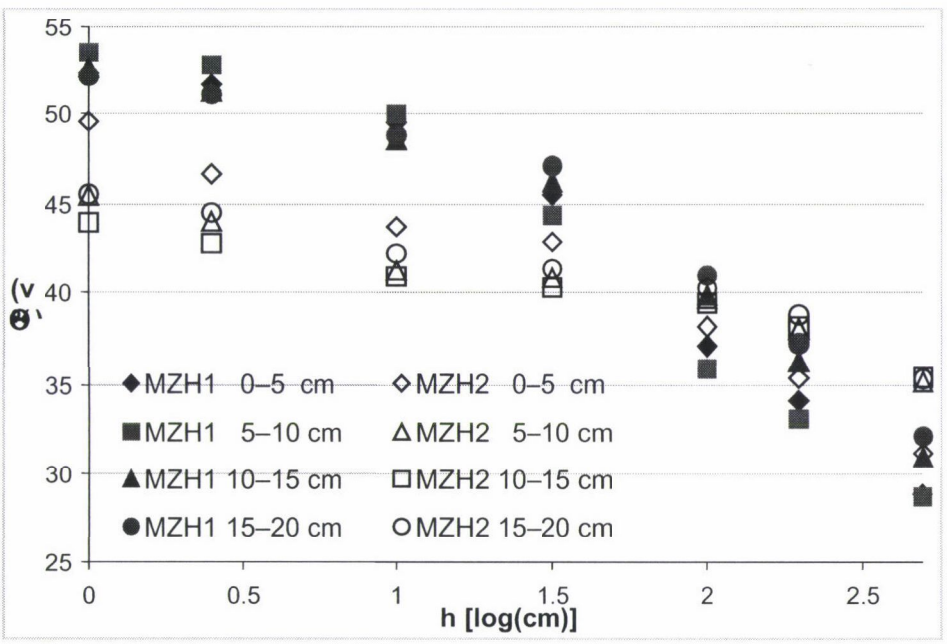

Fig. 4. Soil water retention curves for the Mezőhegyes site

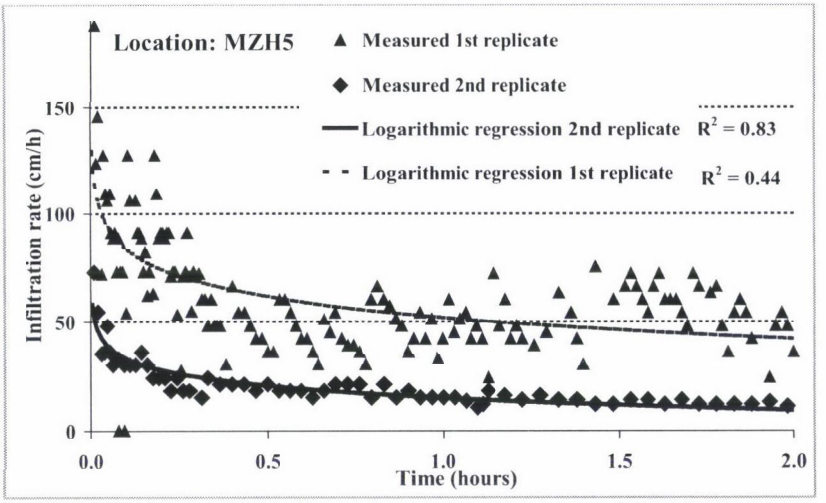

Fig. 5. Infiltration rates measured using double ring infiltrometers at the MZH5 site, and the regression curves fitted to the measured data

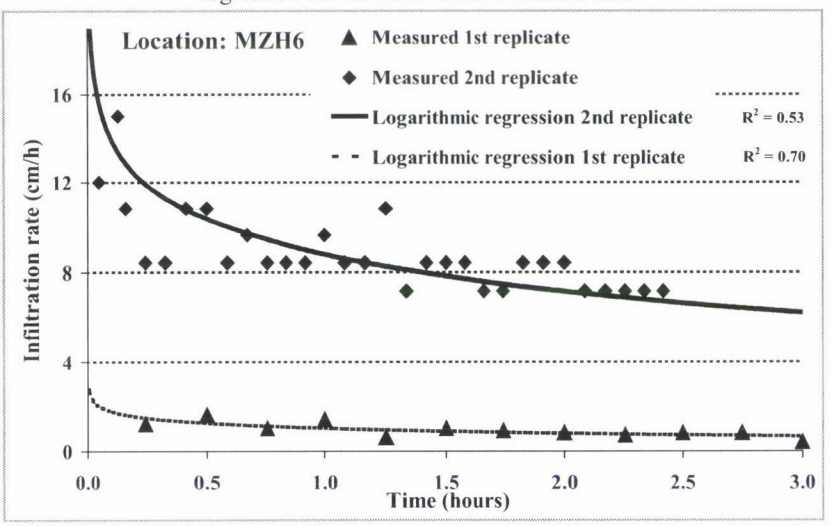

Fig. 6. Infiltration rates measured using double ring infiltrometers at the MZH6 site, and the regression curves fitted to the measured data

Calibration of the TDR 300 instrument

The differences between soil water content values measured with the TDR equipment and those determined from the soil samples were 4 and 0 v% for cylinders with 22 and 25 v% water content, respectively (Table 4). However, the TDR 300 equipment greatly overestimated the Θ values in cylinders with higher soil water contents (by 21–40 v%). Moreover, for the Mezőhegyes soil it indicated larger Θ values in the cylinder with 44 v% water content than in that with 49 v% (Table 4). These results show the uncertainty of the results obtained with the TDR 300 equipment in wet soils.

Table 3
Saturated hydraulic conductivity values (Ks, cm/day) evaluated using the constant head method (lab.) and the double ring infiltrometer method (*in situ*)

	Soil layer	Ks (cm/day)	
	(cm)	Lab.	*In situ*
MZH5	5–10	3.6	288
	25–30	205.5	
	45–50	26.5	
MZH6	5–10	36.2	648.0
	25–30	32.8	
	45–50	170.2	
MZH7	5–10	113.6	
	25–30	16.7	
	45–50	145.0	

Table 4
Soil water content values (v%) measured with TDR 300 equipment, compared to the reference values

Hódmezővásárhely		Mezőhegyes	
Reference values	Measured with TDR 300	Reference values	Measured with TDR 300
21.7	18.0	30.8	52.0
24.8	25.0	36.5	65.0
30.2	52.0	39.4	74.0
36.2	71.0	44.0	84.0
40.6	73.0	49.2	80.0
46.6	75.0	–	–

Small differences of up to 2 and 6 v% for dry and wet soils, respectively, were obtained when soil water content measurements were replicated within each soil column, verifying the reproducibility of the TDR 300 measurements.

The calibration curves for the TDR 300 equipment are given in Figure 7. The R^2 values obtained by fitting a linear regression curve to the measured data were 0.88 and 0.83 for the Hódmezővásárhely and Mezőhegyes soils, respectively. When a second order polynomial regression was fitted to the Hódmezővásárhely data the R^2 value increased to 0.92.

Calibration of the 3T-M capacitance probe

Under laboratory conditions, using soil samples of limited volume, the measurements performed with the 6th probe unit (0–10 cm depth) were inaccurate because of the strong edge effect. Therefore, the evaluation was based on data measured using the 7th and 8th probes. For the Mezőhegyes soils, the Θ values were 34.6 v% for the 10–20 cm layer (7th unit) and 34.6 v% and 34.3 v% for the 20–30 cm layer (8th unit), depending on the soil code (3 for loam and 4 for loamy clay) used in the evaluation software. Thus, the measurement error was less than 1 v%. For the Hódmezővásárhely soil, the measurement error of Θ was 5 v% for the 20–30 cm layer, while it ranged between 1.1 v% and 1.4 v% for the 10–20 cm layer.

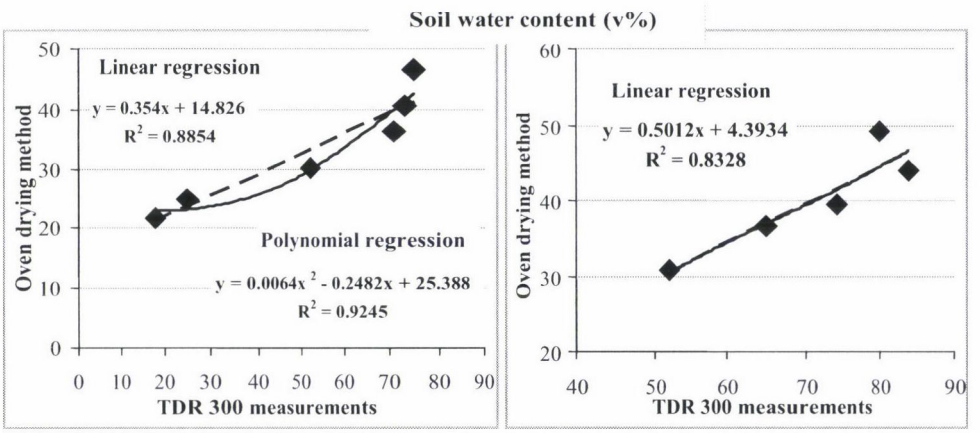

Fig. 7. Calibration curves obtained for the TDR 300 equipment on Hódmezővásárhely (left) and Mezőhegyes (right) soils

The soil water content data measured at the experimental site were compared with the reference data (Table 5). The values recorded with the 3T-M probes were within the ±5 v% accuracy interval acceptable in the case of *in situ* measurements. The greatest deviation between the measured and reference values was found for the 0–10 cm layer, probably because of the side effect and because the variation in soil properties is the highest in this layer. The calibration curve calculated for the capacitance probe using linear regression is given in Figure 8.

Table 5
Soil water content values (v%) measured *in situ* with 3T-M capacitance probes, compared to the reference values

Profiles	Depth (cm)	3T-M	Reference	Difference (3T-M – ref.)
Profile 1	0–10	24.05	28.8	–4.8
	10–20	30.06	30.0	0.1
	20–30	33.07	32.3	0.7
	30–40	33.07	32.0	1.1
	40–50	28.72	30.5	–1.8
Profile 2	0–10	21.61	27.1	–5.4
	10–20	28.09	22.2	5.8
	20–30	26.24	29.8	–3.5
	30–40	29.33	32.9	–3.6
	40–50	26.86	31.7	–4.8

Comparison of the measurement devices

The soil water content values measured using the TDR 300 and 3T-M instruments were compared for both the experimental sites. Figure 9 demonstrates the relationship between the data measured at profiles 1 and 2 in Mezőhegyes. A close correlation was found between the two datasets, though

the TDR instrument overestimated the Θ values. Similar conclusions could be drawn when analysing the soil water retention data: the saturated water content of the soil was at most 51 v%, while values of up to 70 v% were obtained with the TDR 300 equipment. For this reason the data measured using the 3T-M capacitance probe were used for further evaluation.

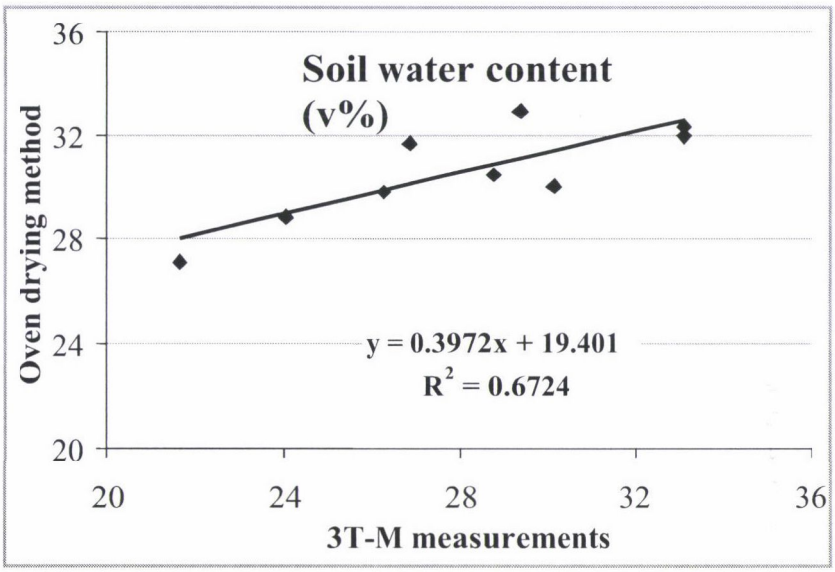

Fig. 8. Calibration curves obtained for the 3T-M capacitance probe

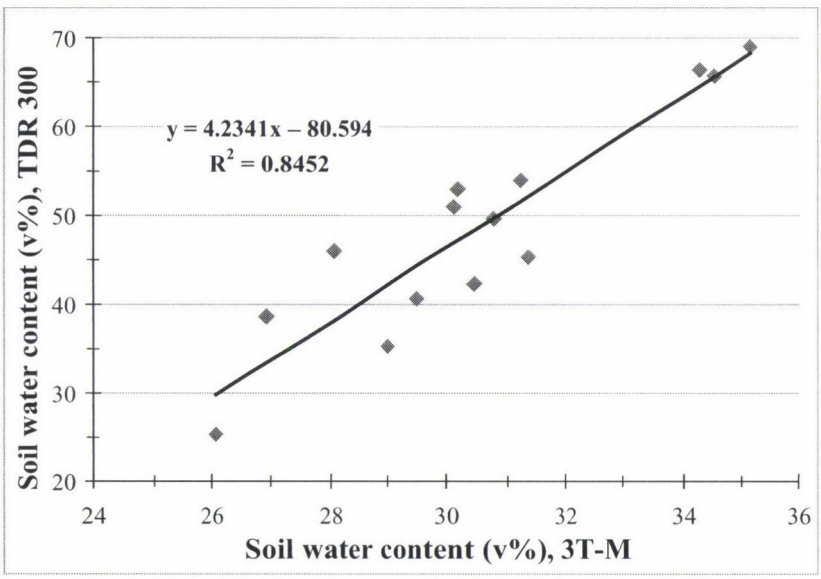

Fig. 9. Relationship between the soil water content values measured with two types of equipment

Evaluation of the soil water content data

The soil water content dynamics measured at the experimental sites during the study period are presented in Figures 10–12. The strongest fluctuation in the soil water content was observed in the upper 20 cm layer. Outstanding differences in Θ were measured at the same time but at different measurement stations, depending on whether the measurements were carried out before or after irrigation. Without irrigation the soil water content would drop below the wilting point in many cases. Despite the intensive evaporation and transpiration, no great changes in Θ were observed below 40 cm. The soil water content below this layer was always above the wilting point. This indicates that sufficient water was available for maize via capillary water rise throughout the vegetation period.

When comparing the total water content of the soil at different locations (Fig. 13), no great differences were found between the soil water regimes of different profiles in the same field. Station MZH3, which had relatively low Θ values, was the only exception. This phenomenon, however, cannot be explained by differences in the soil texture or soil hydrophysical properties of the reference soil profiles. It is assumed that this could be attributed to differences in other factors (such as surface runoff, evaporation conditions, etc.), which influence the elements of the soil water balance in different ways.

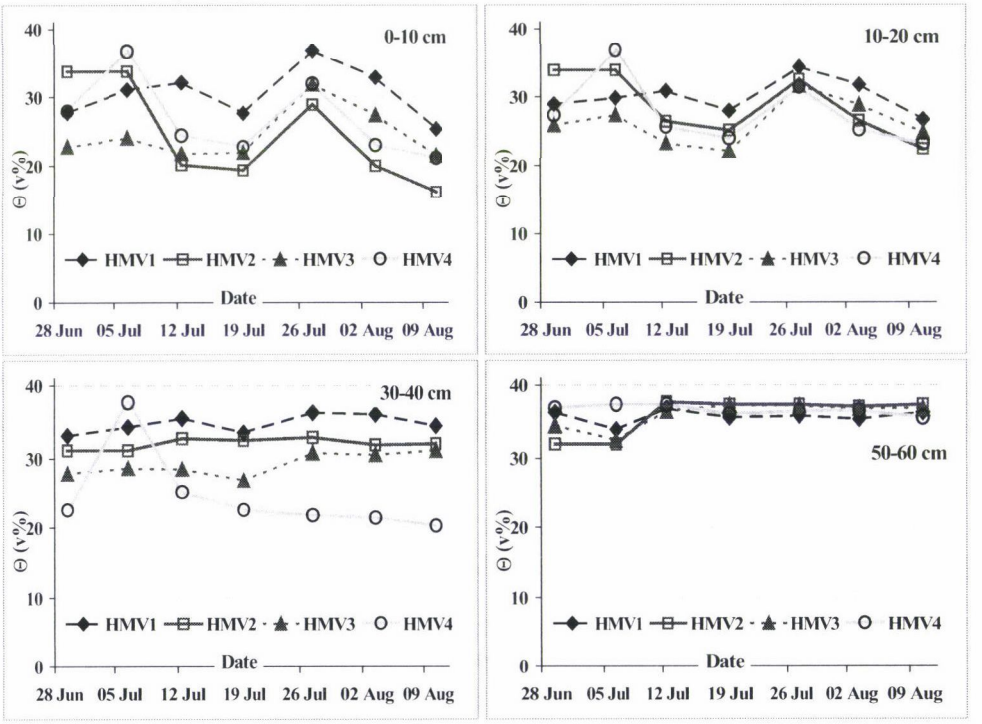

Fig. 10. Soil water content dynamics measured in Hódmezővásárhely in 2003

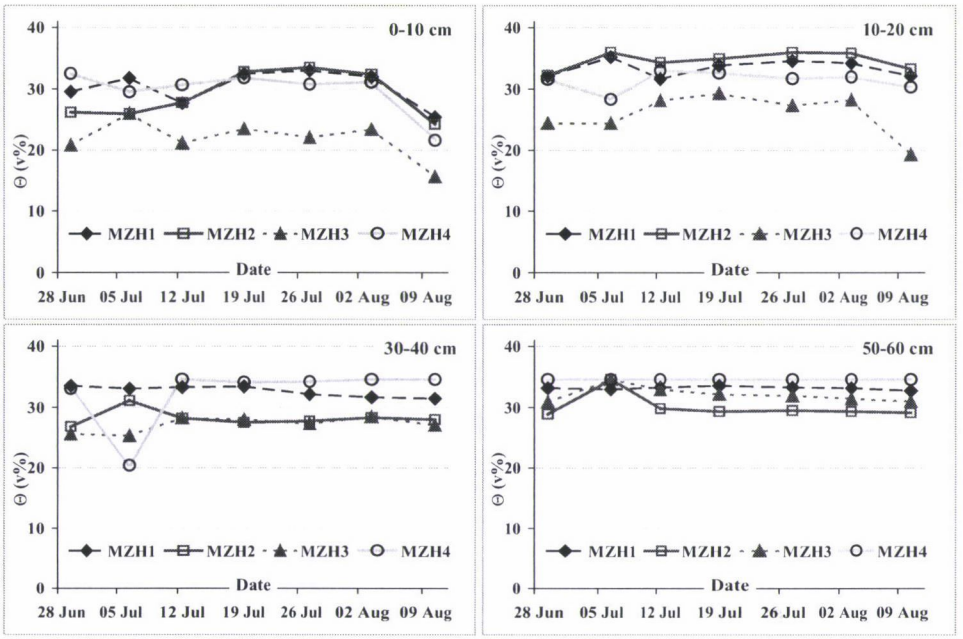

Fig. 11. Soil water content dynamics measured in Mezőhegyes in 2003

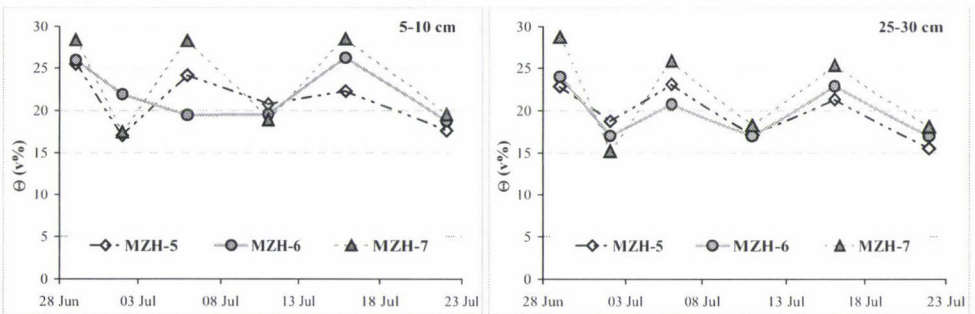

Fig. 12. Soil water content dynamics measured in Mezőhegyes in 2004

Discussion

The soil profiles examined were taken from locations indicated on large soil maps to represent the whole range of variation in soil properties within the fields. However, no significant differences were found in the studied properties. The Hódmezővásárhely and Mezőhegyes fields could be considered to be relatively homogeneous both for physical and hydrophysical properties and for soil water regime. Consequently, monitoring stations established for one or two carefully selected soil profiles could provide enough data to ensure proper decisions on irrigation.

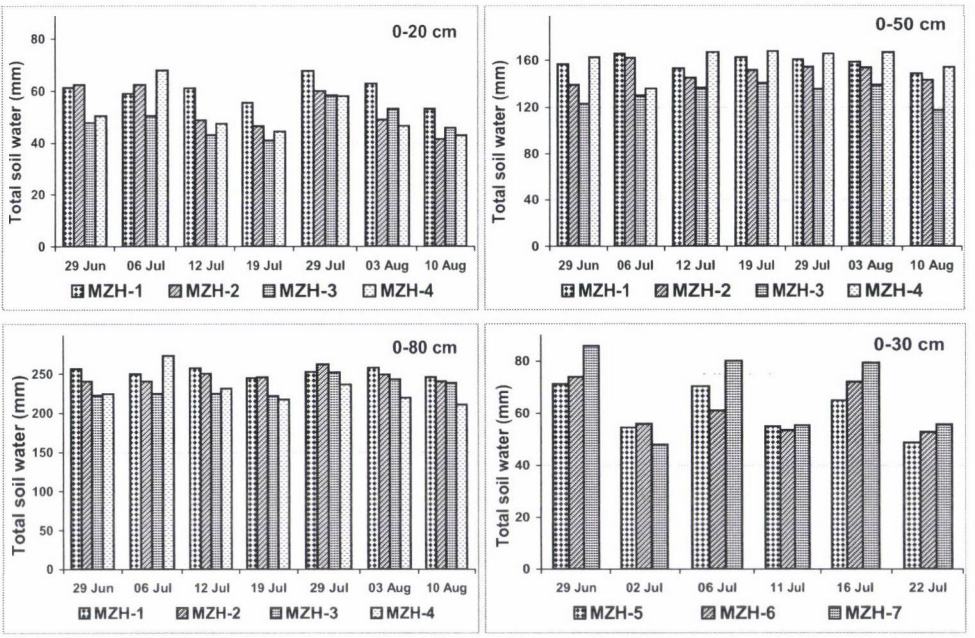

Fig. 13. Total amount of water stored in the Mezőhegyes profiles up to certain depths in 2003 (MZH1–MZH4) and 2004 (MZH5–MZH6)

The equipment tested for the measurement of soil water content, namely the TDR 300 instrument and the 3T-M capacitive probe, needs to be calibrated and tested under field conditions before use. Even though the TDR 300 equipment is widely recommended by the distributors for farmers, it was found that the instrument was difficult to calibrate under certain soil conditions.

Undisturbed soil cores with a volume of 100 cm^3 are too small to represent the macropore flow, which often plays a major role in water redistribution within the soil profile. Kutilek and Nielsen (1994) showed that the volume of the soil sample should be larger or equal to the Representative Elementary Volume (REV) to ensure the independence of the measured soil properties from the choice of sampling site. The smallest value of REV, equal to 100 cm^3, was found for non-structured soils. Due to the disadvantages of the standard constant head laboratory method it was concluded that *in situ* measurements on soil hydraulic conductivity should be strongly recommended. Measurements should be performed on a relatively large soil surface so that the water conductivity of both the soil matrix and the macropores could be detected.

It was found that the total porosity and the saturated hydraulic conductivity of the soil were large and no soil compaction occurred. The soil water content in the rooting zone did not drop below the wilting point and was close to field capacity in the subsoil. These results indicate that the soil management system and irrigation strategy used in the experimental fields ensured satisfactory soil and soil moisture conditions.

Acknowledgements

This research was supported by grants from the Hungarian Ministry of Education (NKFP-4/0008/2002 and NKFP-4/064/2004) and the Hungarian National Scientific Research Fund (OTKA T-042996), and by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences.

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Acta Agronomica Hungarica, 53(2), pp. 177–182 (2005)

STRATEGY FOR IMPROVEMENT OF DOUBLED HAPLOID PRODUCTION IN MAIZE

B. BARNABÁS, T. SPITKÓ, K. JÄGER, J. PINTÉR and L. C. MARTON

AGRICULTURAL RESEARCH INSTITUTE OF THE HUNGARIAN ACADEMY OF SCIENCES, MARTONVÁSÁR, HUNGARY

Received: 10 June, 2005; accepted: 20 July, 2005

In the present study the applicability of a self-constructed doubled haploid line (DH 105) in the *in vitro* breeding of maize was evaluated. This line, which contained only 50% exotic (Chinese) germplasm, could be used to transmit *in vitro* androgenic ability into non-responsive breeding materials. F_1 hybrids resulting from single crosses between the moderately responsive line DH 105 and recalcitrant genotypes with high breeding value showed a considerable heterosis effect in their androgenic responses. Most of the hybrids had favourable morphological and agronomic characters on the basis of "per se" evaluation. The data of the experiments showed that these F_1 hybrid plants could be successfully used as anther donors, since numerous fertile DH plants were developed from their anther cultures. By the use of this *in vitro* breeding strategy the genetic variability can be widened and the effectiveness of inbred line production might be improved.

Key words: anther culture, *in vitro* breeding, maize, *per se* evaluation

Introduction

The heterosis breeding of maize requires a continuous supply of new inbred lines in order to produce hybrids that satisfy market demands. The development of homozygous lines by conventional methods is rather a time-consuming procedure, which is why haploid techniques could be utilised to increase the efficiency of breeding. The potential of doubled haploids in maize breeding has long been recognised (Chase, 1969). Various combinations of genes can be obtained in homozygous form, and then fixed by the use of doubled haploid techniques in a relatively short time. After the pioneering work of Chinese scientists (Kuo et al., 1978; Miao et al., 1978), anther culture was the first promising technique to produce DH plants of microspore origin at satisfactory frequency. Much effort has been made to improve the *in vitro* responsiveness of various maize genotypes and optimise the culture conditions (Genovesi and Collins, 1982; Dieu and Beckert, 1986; Petolino and Jones, 1986). However, genotype dependence is still one of the main limiting factors in the anther culture of maize. Genotypes with high *in vitro* androgenic capacity are of exotic (mainly Chinese) origin, while breeding lines with market value are recalcitrant (Brettel et al., 1981; Pauk, 1985; Barnabás, 2003). The results of genetic studies on the inheritance of haploid induction ability in maize microspores (Murigneux et al., 1994) have shown that this feature is highly heritable. Thus, *in vitro* androgenic ability can be transmitted from exotic sources into elite lines for breeding purposes (Barloy et al., 1989).

0238–0161/$ 20.00

For a number of years, intensive research has been underway in the Martonvásár institute in order to improve the anther culture technology (Barnabás et al., 1998; Obert et al., 1998; Barnabás et al., 1999; Barnabás, 2003) and the efficiency of doubled haploid plant production in maize. The development of genotypes responsive to anther culture, having high haploid induction ability, which can be transmitted into breeding lines with agronomic value, has been a major goal. It is clear from earlier work (Barnabás et al., 2003) that exotic stocks of Chinese origin and their DH offsprings can be used to transfer improved anther culturability into non-responsive elite lines of maize by crossing.

In the present study another DH line of different pedigree and with better agronomic value was examined to evaluate the applicability of the anther culture technology in maize breeding.

Materials and methods

Plant material

The present experiments were carried out on the following maize genotypes: the doubled haploid line DH 105, produced in the experimental nursery of the Martonvásár institute using the anther culture technique from the F_1 hybrid of the Chinese single cross hybrid (CH592×A2) and the inbred line SR88, selected for tolerance to herbicides of the sulphonyl-urea type in Martonvásár, was chosen because of its genetic background and favourable morphological characters. The following local inbred and proprietary lines were used as crossing partners: HMv 5405, B 54/01, 5080/02, 5076/02, B 45/02, TKA 99/Dv, BE 281 and B 52/00. The lines and F_1 hybrids were raised in the experimental nursery of the institute. "Per se" evaluation of the F_1 hybrids was carried out in 2004. The seeds were sown on 23 April. The following traits served as the basis of evaluation: germination, early development, plant height, height of ear attachment, stalk stability, tassel morphology, flowering date and disease resistance.

Anther culture

Tassels were collected for anther culture prior to the emergence of the leaf sheath when the microspores were in the mid-uninucleate stage of development. After collection, the excised young tassels were surface-sterilized with 1% sodium hypochlorite and then packed into aluminium foil and stored at 7°C for 1 week. After this cold pre-treatment, the developmental stage of the microspores was checked again using a light microscope. Parts of the tassels which contained late-uninucleate or early binucleate microspores were sterilized with 2% sodium hypochlorite for 20 minutes and then washed three times in sterile distilled water. The anthers were removed from the florets and inoculated onto the surface of F medium (modified YP medium, Genovesi and Collins, 1982) supplemented with 0.1 mg l^{-1} 2,3,5-TIBA, 5 g l^{-1} activated charcoal, 500 mg l^{-1} casein hydrolysate, 120 g l^{-1} sucrose and 2.5 g l^{-1} gelrite at pH 5.8. The induction medium was autoclaved using the usual protocol. In every genotype 1000–3000 anthers were inoculated.

The cultures (100 anthers in each Petri dish) were incubated in the dark at 29°C for 28 days. After the incubation period the number of responding anthers and the number of induced structures (mainly calli) were counted and harvested. The percentage of anther and callus induction was then calculated.

Plant regeneration

After the induction period the calli were removed from the anthers and transferred directly to a regeneration medium consisting of MS macronutrients, microelements and vitamins

(Murashige and Skoog, 1962). The medium was supplemented with 1 mg l^{-1} kinetin, 0.5 mg l^{-1} NAA (naphthyl acetic acid), 20 g l^{-1} sucrose and 7 g l^{-1} agar at pH 5.8. Plant differentiation was carried out under 16 hours illumination (50 mMol m^{-2} s^{-1} light intensity) at constant 26°C. After 14–16 days the differentiated plantlets were transferred into bigger (200 ml) glass containers for further growth, using half the concentration of the same MS medium. Two weeks later the well-developed plantlets were transplanted into peat pellets 5 cm in diameter (AS Jiffy Products Ltd.) and covered with plastic film for about 5 days to maintain high relative humidity. Finally the regenerants were transplanted to soil in 10 l plastic pots and grown in phytotron chambers according to the "Bk" programme elaborated by Barnabás and Rajki (1976) for the phytotronic cultivation of maize (Tischner et al., 1997). Fertile plants, which had normal, functionable male and female sexual organs, were self-pollinated. Maize plants where only the female or the male inflorescences developed normally, were "sib"-pollinated using a sister plant of the same culture as a crossing partner.

Results and discussion

The DH 105 line (which has only 50% exotic background) showed medium/low responsiveness to anther culture (Table 1). However, if this doubled haploid line was crossed with non-responsive inbred lines, a considerable heterosis effect could be observed in the induction parameters of the F_1 hybrids (Table 2). These data support earlier findings (Barnabás, 2003; Barnabás et al., 2003) where the same tendency was detected in anther cultures of F_1 hybrids originating from crosses between a highly responsive DH line of 100% exotic (Chinese) origin and recalcitrant commercial lines. This indicates that *in vitro* androgenic ability could be developed in the hybrids and that hybrid vigour can be manifested not only at the plant (sporophyte) level, but also in haploid microspores cultured *in vitro* (gametophytes).

Apart from the strong genotype dependence, the number of fertile, doubled haploid offspring that can be obtained depends on numerous external and internal factors, such as the *in vitro* culture environment for plant regeneration, abiotic environmental conditions for planting and further plant growth, and the ploidy level and vitality (physiological condition) of the plants. This could have been the reason for the variable number of normal, fertile DH0 plants finally produced (Table 2).

These results underline reports by Murigneux et al. (1994) that haploid induction ability is a heritable character. The additive nature of *in vitro* androgenic ability (Petolino and Thompson, 1987) made it possible to introduce this trait into recalcitrant genotypes via conventional crossing. One important question is the extent to which the favourable agronomical traits of commercial lines would be impaired by the presence of exotic germplasm in hybrid-derived DH plants. To answer this question, work has begun on the examination and evaluation of the most important morphological and agronomic features of anther donor plants derived from various crosses.

Table 1
Responsiveness of the line DH 105 in anther culture

No. of anthers cultured	Anther induction %	Callus induction %	Green plant regeneration %*	No. of fertile plants
1000	1.5	2.3	5.0	3

*Per 100 inoculated anthers

Table 2
In vitro androgenic ability of SC hybrids between DH 105 and various breeding lines

Crosses (♀×♂)	No. of inoculated anthers	Induction of anthers[1] (%)	Induction of calli[1] (%)	Regeneration of green plants[1] (%)	No. of fertile plants grown to maturity		
					Total	Sib-poll.	Self-poll.
HMv5405 × DH 105	3000	9.8	21.6	3.2	50	12	27
DH 105× HMv5405	1500	38.8	96.9	17.8	59	10	21
DH 105 × B54/01	2900	12.4	27.1	4.1	45	16	17
B54/01 × DH 105	1000	14.4	44.4	3.9	14	–	3
DH 105 × 5080/02	2500	5.8	13.8	3.1	30	14	11
DH 105 × 5076/02	1900	3.0	6.7	2.1	16	7	6
DH 105 × B45/02	1000	3.9	7.9	1.1	5	1	4
DH 105 × TKA 99/Dv	3000	1.0	1.5	0.3	5	2	2
DH 105 × BE 281	1000	3.0	4.1	0.6	2	–	1
DH 105 × B52/00	2500	3.4	6.9	0.7	5	1	1

[1]Per 100 inoculated anthers

The tasselling date is one of the most important traits from the breeding point of view. In Hungary only genotypes that finish flowering by the end of July are taken into consideration by breeders.

Since the mean flowering date of the genotype DH 105 is during the last ten days of July, the inbred lines chosen as crossing partners must flower at the same time (Table 3).

When the lines were compared with the F_1 hybrids with regard to the time required for 50% tasselling, it could be seen that approx. 50% of the single cross hybrids showed intermediate inheritance, but in one case (B 54/01 × DH 105) this period was longer than that of either parent, while in four other hybrids (DH 105 × 5080/02, DH 105 × 5076/02, DH 105 × B 45/02 and DH 105 × BE 281/00) it was significantly shorter (Table 3).

The "per se" evaluation of the F_1 hybrids (Table 4) showed that most of them possessed favourable (4–4.5 on a 1–5 scale) morphological traits and other agronomically important characters.

The use of new hybrids as anther donor plants could serve to widen the genetic basis for the doubled haploid breeding of maize.

Table 3
Comparison of the time to tasselling in parental lines and F_1 hybrids

Lines		Hybrids	
Genotype	Days to 50% tasselling	Crosses	Days to 50% tasselling
DH 105	93		
HMv 5405	87	HMv 5405 × DH 105	88
		DH 105 × HMv 5405	91
B 54/01	88	DH 105 × 54/01	91
		B 54/01 × DH 105	96 ↑
5080/02	94	DH 105 × 5080/02	85 ↓
5076/02	94	DH 105 × 5076/02	78 ↓
B 45/02	86	DH 105 × B45/02	82 ↓
BE 281/00	94	DH 105 × BE281/00	86 ↓
B 52/00	86	DH 105 × B52/00	86
TKA 99/Dv	96	DH 105 × TKA 99/Dv	96

Table 4
"Per se" evaluation of the F_1 hybrids

Genotype	Average score*
HMv 5405 × DH 105	4.2
DH 105 × HMv 5405	4.5
DH 105 × B 54/01	4.0
B 54/01 × DH 105	3.5
DH 105 × 5080/02	4.6
DH 105 × 5076/02	4.5
DH 105 × B 45/02	4.5
DH 105 × TKA 99/Dv	3.2
DH 105 × BE 281/00	3.0
DH 105 × B 52/00	4.4

* 1 unfavourable; 5 very good

Although considerable progress has been made over the last two decades in improving the *in vitro* androgenic response by optimising the anther culture conditions, the very low response frequency in all but a few (mainly exotic) genotypes still remains the major problem in maize doubled haploid breeding. As superior maize genotypes are rather recalcitrant to anther culture in general, the production of a sufficient number of homozygous doubled haploid lines in one generation is difficult.

The present data demonstrate that the *in vitro* androgenic response could be successfully transferred into hybrids from a single cross between the moderately responsive doubled haploid line DH 105 and genotypes with high commercial value, and that the androgenic response of the hybrids exhibited heterosis, i.e. it exceeded that of both parental genotypes.

However, differences can still be observed in the number of microspore-derived structures and regenerants in given genotypes. The correlation between the chromosomal changes in microspore-derived structures during the induction period and their regeneration potential still needs to be explained.

Acknowledgements

This work was supported by grant No. OM NKFP 137/2002 from the Ministry of Education. The authors would like to thank Erika Gondos for carrying out the field experiments.

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Acta Agronomica Hungarica, 53(2), pp. 183–196 (2005)

INVESTIGATIONS ON THE ADAPTABILITY OF MAIZE LINES AND HYBRIDS TO LOW TEMPERATURE AND CADMIUM

G. SZALAI, M. PÁL, E. HORVÁTH, T. JANDA and E. PÁLDI

AGRICULTURAL RESEARCH INSTITUTE OF THE HUNGARIAN ACADEMY OF SCIENCES, MARTONVÁSÁR, HUNGARY

Received: 10 June, 2005; accepted: 20 July, 2005

In the course of the Maize Consortium Project, investigations were made on the defence mechanisms employed by maize against various abiotic stress factors (low temperature, cadmium) and on the effects exerted by two compounds (S-methylmethionine, salicylic acid) capable of improving the stress resistance of plants to certain abiotic stresses. Salicylic acid (SA) was found to inhibit the uptake of cadmium (Cd), but caused damage to the roots, including a reduction in the activity of phytochelatin synthase (PCS), which meant that preliminary treatment with SA aggravated the damaging effect of Cd. It was also proved that as the result of 2-day treatment with Cd, there was a continuous rise in the Cd level in the plants, more Cd being accumulated in young leaves than in older ones. The PCS activity increased greatly after 24 hours, both in the leaves and in the roots, declining again after 2 days. The effect of SA was examined in both the hybrids and their parental lines, and the effect of this compound on the intensity of alternative respiration was also investigated. A comparison of chilling tolerance data and antioxidant enzyme activity indicated that these two parameters were not directly correlated to each other, i.e. antioxidant enzyme activity values could not be used to draw reliable conclusions on the chilling tolerance of maize lines and hybrids. With regard to the interaction between alternative respiration and salicylic acid, it was proved that exogenous hydrogen peroxide caused a similar increase in the ratio of alternative respiration to that observed after salicylic acid treatment.

Abbreviations: SA, salicylic acid; Cd, cadmium; PCS, phytochelatin synthase; SMM, S-methylmethionine; PCs, phytochelatins; PAR, photosynthetically active radiation; TTC, triphenyl tetrazolium chloride; KCN, potassium cyanide; PSII, 2nd photochemical system; POD, guaiacol peroxidase; APX, ascorbate peroxidase; GR, glutathione reductase

Key words: maize, chilling tolerance, alternative respiration, chlorophyll-a fluorescence induction, antioxidant enzymes, phytochelatin, phytochelatin synthase, S-methylmethionine, ion leakage

Introduction

The damaging effect of cold weather on maize, one of the most important crops, is generally experienced during the early stages of plant development, both in Hungary and in many other countries of the world. In plants the effect of low temperature and its duration can be detected by investigating photosynthesis, free amino acids, polyamines, etc. Plants such as maize stop growing at temperatures below 5–7°C, and may even die after a certain length of time due to the increased oxidative stress at low temperature. Many authors have

0238–0161/$ 20.00

proved the role of antioxidant and radical-scavenging enzymes in protecting plants from the cold. It was demonstrated that exogenously applied superoxide and singlet oxygen radical scavengers reduced the membrane damage caused by low temperature. It can be stated on the basis of these results that the extent of oxidative damage suffered as the result of cold stress depends on the antioxidant capacity of the plant. One aim of the present work was to compare the chilling tolerance and antioxidant capacity of maize hybrids and their parental inbred lines. Investigations were also made on changes in the alternative respiration pathway as the result of treatments influencing the chilling tolerance of maize (salicylic acid and hydrogen peroxide).

One of the main aims of research on plant stress physiology has always been to investigate the metabolism of compounds capable of increasing the defence capacity of plants against abiotic stress. One such compound, S-methylmethionine (SMM) is able to intensify the biosynthesis of proteins, nucleic acids and chlorophyll. Observations have also suggested that SMM may be involved in the biosynthesis of polyamines, which have an important role in biological regulation (Giovanelli et al., 1980; Dethier et al., 1991; Lásztity et al., 1997; Gyetvai et al., 2002). On the basis of previous results, the present aim was to determine the extent to which SMM was capable of protecting membrane integrity during chilling stress, i.e. of reducing membrane damage at low temperature. Certain heavy metals, such as cadmium, may be introduced into the soil with mineral fertilisers (Dániel and Győri, 2000). The problem of heavy metal stress is aggravated by the fact that, while low temperature only causes damage to the plant, the accumulation of heavy metal in the plant may cause toxicity in humans, either indirectly (when the plants are used as fodder for farm animals) or directly (when the plants are used for human consumption). Phytochelatins (PCs), which are able to bind heavy metals to various extents, are an important part of the plant defence system (Cobbet, 2000). Although the gene coding for phytochelatin synthase (PCS) has already been isolated from wheat (Clemens et al., 1999), few data are available for monocots. As cadmium is known to cause oxidative stress in plants (Hegedűs et al., 2001), one aim of the present research was to examine how treatment with salicylic acid (SA), a compound proved to protect plants against certain abiotic stress factors, such as heat stress and low temperature (Dat et al., 1998; Janda et al., 1999), influenced cadmium stress under conditions where SA was able to provide protection against chilling damage.

Materials and methods

Plant material

Various maize hybrids and inbred lines [H1: L5×L7; H2: L3×L4; H3: L5×L2; H4: L1×L2; H5: (L8×L9)×L4; H6: L5×L6] were grown in Conviron growth units in the phytotron of the Agricultural Research Institute in Martonvásár under controlled conditions (light, temperature, humidity) in a 3:1:1 mixture of garden soil, Vegasca (a humus-containing additive manufactured by Florasca Ltd., Hungary) and sand in 9 cm Ø pots or in beakers containing 250 ml Hoagland solution. Chilling treatment was carried out in the same chamber at 5°C.

In the cadmium treatments, sterilised maize seeds (*Zea mays* L., hybrid Norma) were germinated at 26°C for 4 days, then grown in Hoagland solution for 10 days in a Conviron PGV-36 growth chamber under the following conditions: 16 h illumination, 22/20°C day/night temperature, 200 μmol m^{-2} s^{-1} light intensity, 75 % relative humidity.

Some of the plants were treated with 0.5 mM $Cd(NO_3)_2$ (1dCd) or 0.5 mM SA (1dSA) for 1 day. Others received a combination of Cd and SA, applied consecutively (1dSA+1dCd) or together (1d[SA+Cd]). In the 1dSA+0 treatment no Cd treatment was applied after the preliminary SA treatment, but the plants were grown for a further day.

Chlorophyll fluorescence induction measurements

Chlorophyll fluorescence induction parameters were measured on the youngest fully developed leaves using a PAM-2000 fluorometer (Walz, Effeltrich, Germany) at room temperature. The plants were dark-adapted for 30 min prior to measurement. Maximum fluorescence was determined using a 0.8 s flash of white light (PAR > 3000 μmol m^{-2} s^{-1}) and the results were evaluated using the DA-2000 measurement program. The nomenclature suggested by van Kooten and Snel (1990) was used for the chlorophyll fluorescence induction parameters.

Enzyme extraction

One g plant material was ground with 0.5 g quartz sand in 0.5 mM TRIS-HCl buffer (pH 7.4) containing 1 % PVPP at 4°C. The homogenate was filtered through 4 layers of gauze, then centrifuged in a chilled centrifuge (20 min, 12000 g). The supernatant was divided into Eppendorf tubes and stored at –20°C until measurement.

Enzyme activity measurements

The total protein concentration of the samples was determined using Bio-Rad reagent, based on the method described by Bradford (1976). The absorbance of the reaction mixture was recorded with a spectrophotometer at 595 nm. Enzyme activities were determined photometrically (Shimadzu UV-VIS 160A), except for tests on the SA inhibition of the catalase enzyme, which were carried out using an oxygen electrode (CB1D, Hansatech Ltd., UK). The samples were kept on ice until measurement, while the measurements were carried out at room temperature.

Catalase (EC 1.11.1.6) activity was recorded at 240 nm by tracing the hydrogen peroxide consumption. The total volume of the reaction mixture was 3 ml, and contained 10 mM H_2O_2 and 50 μl plant sample in 0.5 mM TRIS buffer (pH 7.4) (Ádám et al., 1995).

The activity of guaiacol peroxidase (EC 1.11.1.7) was determined according to the method of Ádám et al. (1995) by measuring the increase in absorbance at 470 nm in the course of guaiacol oxidation. The reaction mixture (3 ml) contained 50 μl sample, 10 mM H_2O_2 and 1 mM guaiacol in 0.1 mM acetate (pH 5.5) buffer.

Ascorbate peroxidase (EC 1.11.1.11) activity was recorded in TRIS buffer (0.2 mM, pH 7.8) containing 25 mM ascorbic acid and 0.5 mM H_2O_2, 50 μl sample being added to 2.25 ml of the reaction mixture. The consumption of ascorbic acid was traced at 290 nm (Nakano and Asada, 1987).

Glutathione reductase (EC 1.6.4.2) activity was determined by measuring the reduction of DTNB at 412 nm, caused by the GSH produced during enzyme activity. The reaction mixture contained 50 μl sample, 0.1 M phosphate buffer (pH 7.5), 1 mM EDTA, 0.75 mM DTNB, 0.1 mM NADPH and 1 mM GSSG (Smith et al., 1988) in a total volume of 1 ml.

Other measurements

The intensity of alternative respiration was determined by recording the oxygen consumption of 3 root pieces, each measuring 2 cm, in 0.1 mM phosphate buffer (pH 6.5) with the aid of an oxygen electrode. The rate of oxygen consumption was recorded first without inhibitor, then in the presence of 15 mM salicyl hydroxamic acid (SHAM), and finally in the joint presence of 0.5 mM KCN and 15 mM SHAM (Moller et al., 1988). KCN is the inhibitor of cytochrome respiration and SHAM that of the alternative respiration pathway.

Root viability was measured by incubating 0.1 g root in 50 mM phosphate buffer (pH 7.5) containing 1.5 ml 0.8% triphenyl tetrazolium chloride (TTC) for 1 day. The roots were then transferred to 3 ml ethanol and kept at 60°C for 30 min. Ethanol absorption was recorded at 485 nm.

The cadmium content was determined using the technique described by Hegedűs et al. (2001), and the activity of phytochelatin synthase using the method reported by Chen et al. (1997). The extent of electrolyte leakage was recorded using the method of Szalai et al. (1996) in an Automatic Seed Analyser capable of analysing 100 samples simultaneously.

Statistical analysis

The results reflect the means of at least 5 replications. Significance was calculated using the T-test method and analysis of variance (ANOVA).

Results

Ability of maize to adapt to cadmium

Chlorophyll-a fluorescence induction

The $\Delta F/F_m'$ chlorophyll-a fluorescence induction parameter characteristic of the light energy absorbed by the 2nd photochemical system (PSII) (Genty et al., 1989) was recorded as a demonstration of the damaging effect of 0.5 mM Cd treatment (Fig. 1).

This value declined as the result of the 1dCd treatment. A 1-day treatment with SA alone (1dSA) did not cause any substantial change, but if the plants were examined a day later (1dSA+0), a reduction in the efficiency of PSII was observed. The most pronounced change was recorded in the 1dSA+1dCd treatment, while the 1d[SA+Cd] treatment had very little influence on the $\Delta F/F_m'$ value.

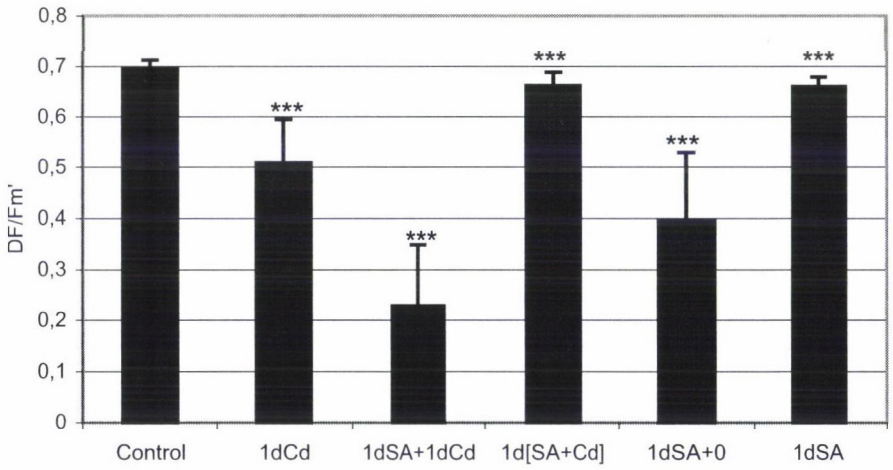

Fig. 1. Effect of 1-day treatments with cadmium (Cd), salicylic acid (SA) or various combinations of these on the quantum efficiency of PSII in young maize (*Zea mays* L., hybrid Norma) plants
***: Differences significant at the P = 0.001 level

Root viability

There was a substantial reduction in root viability after both the 1dSA and 1dCd treatments. However, when applied together or consecutively, SA and Cd had an even greater effect.

Cadmium content

As was expected, Cd accumulated chiefly in the roots. The Cd content was considerably lower in plants given the 1dSA+1dCd or 1d[SA+Cd] treatments (Fig. 2).

Antioxidant enzyme activities

The 1dCd treatment had little effect on the activities of antioxidant enzymes in the roots. The 1dSA treatment, on the other hand, led to an increase in the activity of guaiacol peroxidase (POD) in the roots. When Cd and SA were applied simultaneously, Cd inhibited the increase in POD activity, but Cd applied a day after SA treatment was unable to influence the increased POD activity.

The ascorbate peroxidase (APX) activity increased as the result of all the SA treatments. The joint application of SA and Cd had a synergistic effect, resulting in a 3-fold increase in activity.

The activity of glutathione reductase (GR) increased to the same extent following the 1dSA and 1d[SA+Cd] treatments, but dropped to almost the control level in the 1dSA+0 treatment. In the 1dSA+1dCd treatment the GR activity remained at a high level.

The catalase activity was very low in the roots and did not exhibit any substantial change after Cd and/or SA treatment.

In the leaves, the POD activity was not influenced by any of the treatments. For catalase and APX only the 1dSA treatment caused a significant reduction, while in the 1dSA+0 treatment the enzyme activity returned to the control level. The GR activity increased to the same extent in all the treatments.

Phytochelatins

Phytochelatins (PCs) are enzymatically synthesised, cysteine-rich peptides capable of forming chelates with heavy metals, thus reducing their damaging effect (Grill et al., 1989). The PC level in the roots increased after the 1dCd treatment, but the other treatments had very little influence on the PC level (Fig. 3).

The activity of PC synthase (PCS) in the roots decreased in all the treatments compared with the control (Fig. 4), but the activities were lower in those preliminarily treated with SA than in those treated with SA and Cd together.

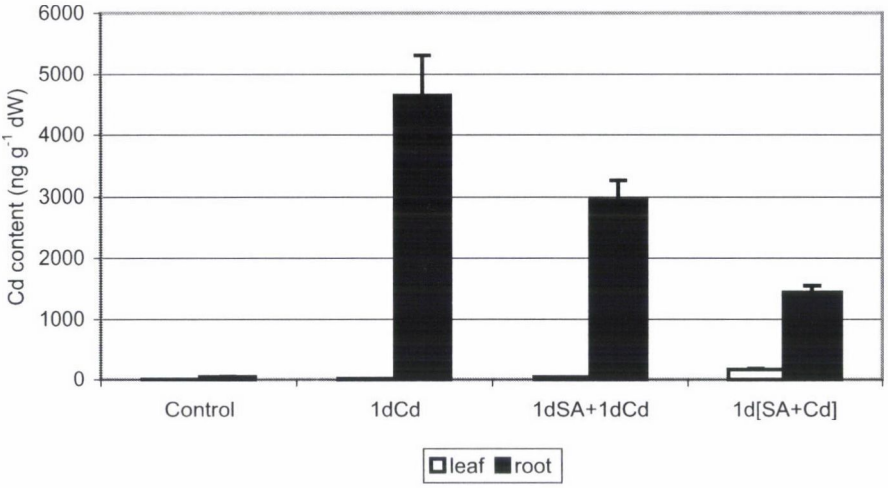

Fig. 2. Changes in cadmium content in the leaves and roots of maize (*Zea mays* L., hybrid Norma) after 1-day treatments with cadmium (Cd), salicylic acid (SA) or various combinations of these

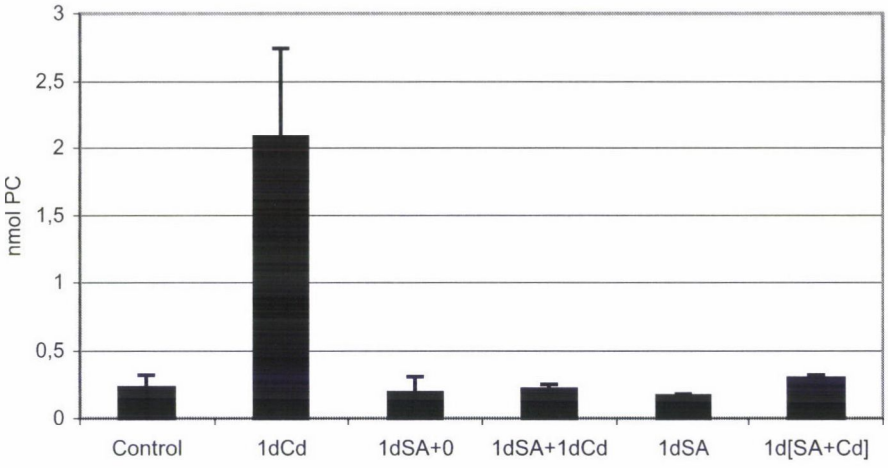

Fig. 3. Changes in the *in vivo* phytochelatin level in the roots of maize (*Zea mays* L., hybrid Norma) after 1-day treatments with 0.5 mM cadmium (Cd), 0.5 mM salicylic acid (SA) or various combinations of these

The PC level in the leaves did not change significantly in any of the treatments (Fig. 5), while the PCS activities increased in all the treatments compared with the control, especially in the case of 1d[SA+Cd] (Fig. 6).

These results suggest that SA may inhibit the uptake of Cd, but causes damage to the roots, including a reduction in PCS activity, with the consequence that preliminary treatment with SA aggravates the damaging effect of Cd.

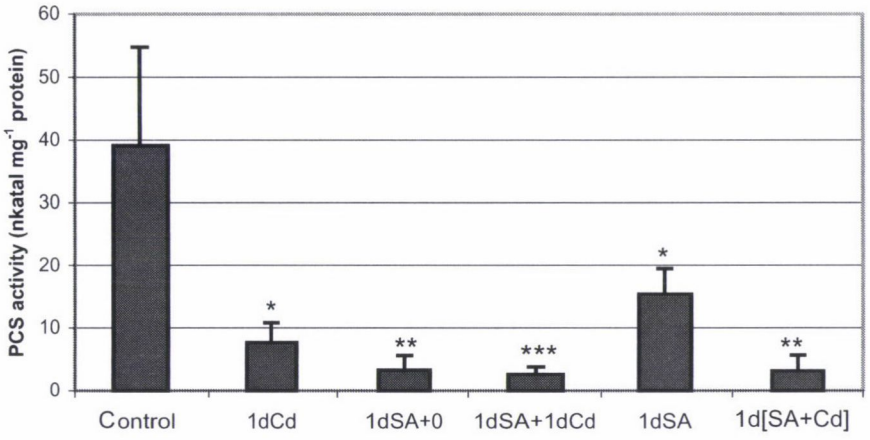

Fig. 4. Changes in the phytochelatin synthase activity in the roots of maize (*Zea mays* L., hybrid Norma) after 1-day treatments with 0.5 mM cadmium (Cd), 0.5 mM salicylic acid (SA) or various combinations of these; *,**,***: Differences significant at 0.1, 0.01 and 0.001 levels, respectively

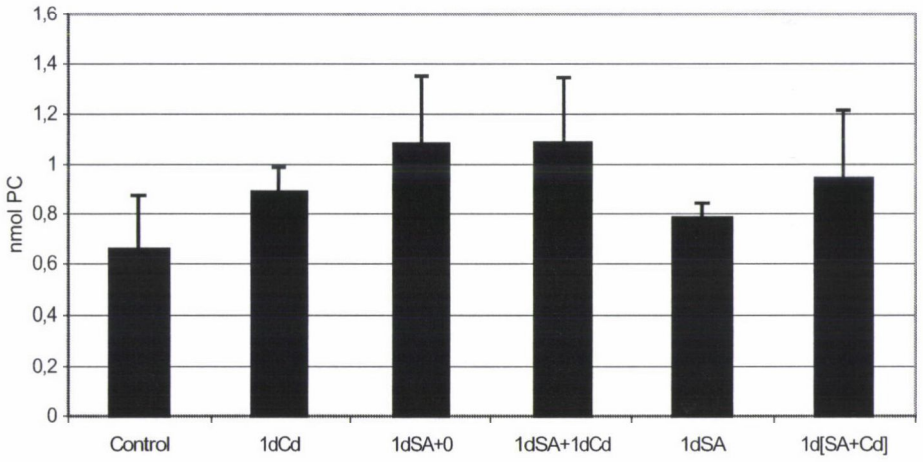

Fig. 5. Changes in the *in vivo* phytochelatin level in the leaves of maize (*Zea mays* L., hybrid Norma) after 1-day treatments with 0.5 mM cadmium (Cd), 0.5 mM salicylic acid (SA) or various combinations of these

Studies on the chilling tolerance and antioxidant capacity of maize hybrids and their parental inbred lines

Six maize hybrids and their parental lines were raised in the phytotron at 22/20°C for three weeks, followed by chilling treatment at 5°C. In order to estimate chilling tolerance the F_v/F_m chlorophyll-a fluorescence induction parameter was measured prior to chilling treatment, after 1, 2 and 7 days of chilling treatment, and after a 1-day recovery period (Fig. 7).

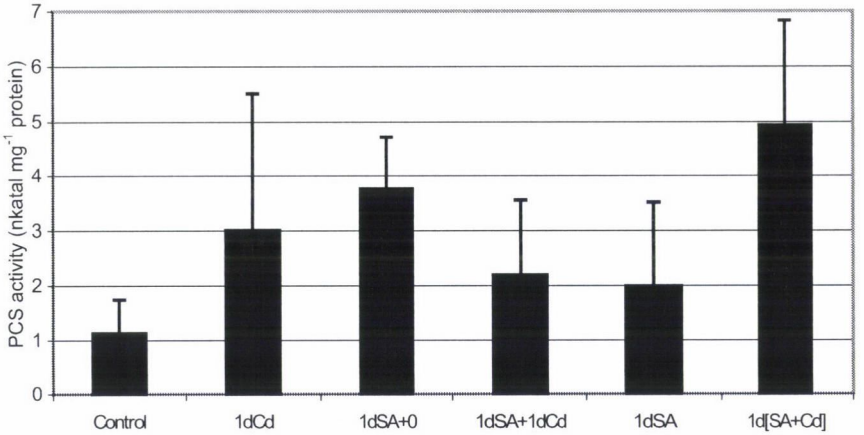

Fig. 6. Changes in the phytochelatin synthase activity in the leaves of maize (*Zea mays* L., hybrid Norma) after 1-day treatments with 0.5 mM cadmium (Cd), 0.5 mM salicylic acid (SA) or various combinations of these

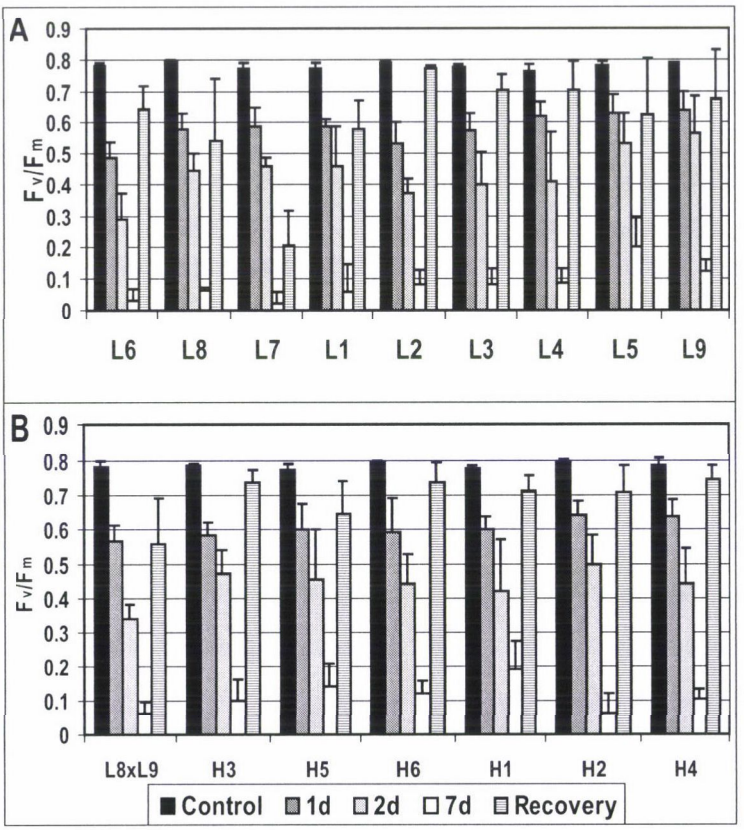

Fig. 7. Changes in the F_v/F_m values of maize (*Zea mays* L.) genotypes after 1, 2 or 7 days of chilling at 5°C and after 1 day of recovery. A: parental lines; B: hybrids

In all cases, the control values were around 0.8, the value characteristic of healthy plants. Following chilling stress this value gradually declined. In order to classify the genotypes, the order of the F_v/F_m values was determined at all the measuring dates. The final order was compiled from the average of these individual orders. On this basis, the lines were divided into groups with good (L5, L9), moderate (L1, L2, L3, L4) and poor (L6, L7, L8) chilling tolerance. The chilling tolerance values recorded for the hybrids did not differ significantly, the only exception being the L8×L9 hybrid, which had poorer chilling tolerance than the other hybrids.

Antioxidant activity of hybrids and their parental lines

The antioxidant activity of the six hybrids and their parental inbred lines was recorded after 6 weeks of growth under control conditions and after a subsequent 1-day chilling treatment at 5°C. The activities of five detoxifiying enzymes were measured. The catalase activity did not change substantially after 1 day of chilling stress in any of the genotypes, with the exception of the hybrid H2. Among the genotypes, lines L2, L4 and L5 were found to have relatively high activity, while that of L3 was extremely low. Among the hybrids, H3 exhibited the highest value, probably due to the high catalase activities recorded for the parental lines. The glutathione reductase activity increased as the result of chilling treatment in all the inbred lines except L9, exhibiting the highest values in lines L2, L4, L7 and L8. As in the case of catalase, line L3 had extremely low activity. Among the hybrids, the activity increased substantially in H5, H1 and H4, but the greatest activity was observed for the genotype L8×L9. The highest level of ascorbate peroxidase activity was recorded for lines L1 and L4. Increases were observed as the result of chilling treatment in lines L1, L4, L6 and L7 and in hybrids H3, H5, H1, H4 and H2. The activity in the latter was far higher than in the other hybrids, presumably because of the high value in the parental line L4. There was only an increase in guaiacol peroxidase activity in line L4 and, to a lesser extent, in the hybrids H1 and L8×L9. The greatest activity was exhibited by genotype L1. Although there was no substantial change in the activity of glutathione-S-transferase in any of the inbred lines, relatively high values were exhibited by L1, L5 and especially L4. A slight increase was recorded in the hybrids L8×L9, H5, H1, H2 and H4.

A comparison of the chilling tolerance and antioxidant data indicates that there is no direct correlation between the two parameters, so no reliable conclusions on the chilling tolerance of maize lines or hybrids can be drawn from the values of antioxidants.

Effect of SA on the intenstiy of alternative (cyanide-resistant) respiration in maize

It has been demonstrated in many plants that salicylic acid increases the intensity of alternative respiration through the induction of the alternative oxidase (Rhoads and McIntosh, 1992). The other frequently investigated effect of SA is the inhibition of the catalase enzyme. Some authors consider that the effect of SA is mediated by the increased hydrogen peroxide level caused by the inhibition of catalase activity (Chen et al., 1993).

The investigations were aimed at determining whether there was any change in the intensity of alternative respiration when maize plants were treated with exogenous SA or hydrogen peroxide. Changes in the intensity of alternative respiration in various treatments were recorded in the roots of 2-week-old maize plants (hybrid Norma) grown in nutrient solution. Treatment involved a 1-day treatment with 0.1 mM salicylic acid or 10 mM H_2O_2. The two treatments had a similar effect (Fig. 8). There was a considerable increase in the intensity of alternative respiration as the result of the treatments, from 25% of the total respiration to 33–35%. The ratio of cytochrome respiration declined to an even greater extent, from 58% to 42 and 40%, respectively, in the two treatments, indicating an increase in the intensity of residual respiration within the total respiration.

In the next stage of the work, alternative respiration was compared in two maize genotypes, the chilling-sensitive Mo17 and the chilling-tolerant CM7. The ratio of alternative respiration in the roots was measured at control temperature (22°C) and during chilling treatment (12°C). Only a slight difference was recorded between the two genotypes at 22°C (Fig. 9), with a higher ratio of alternative respiration in the more chilling-tolerant CM7. At low temperature, however, there was a pronounced difference between the two genotypes. While in the chilling-sensitive Mo17 there was practically no change in the ratio of alternative respiration during chilling treatment, a substantial increase was recorded (from 28% to 39%) in CM7.

Effect of S-methylmethionine on the electrolyte leakage induced by chilling stress

The effect of SMM on the chilling tolerance of young maize plants was tested under phytotron conditions. These experiments were aimed first at determining the extent to which SMM was capable of protecting the membrane integrity of maize leaves at low temperature, and secondly at establishing the correlation between SMM and various photosynthetic parameters. The extent of membrane destruction was traced by recording electrolyte leakage. Various inbred maize lines responding differently to low temperature were tested, but only the results obtained for CM7 will be presented here.

It is clear from Figure 10 that the level of electrolyte leakage due to membrane destruction increased at low temperature. In line CM7, however, this level was significantly lower in plants treated with SMM. This can be explained by the membrane stabilising effect of SMM. Similar results were obtained for the other inbred maize lines studied in the present work.

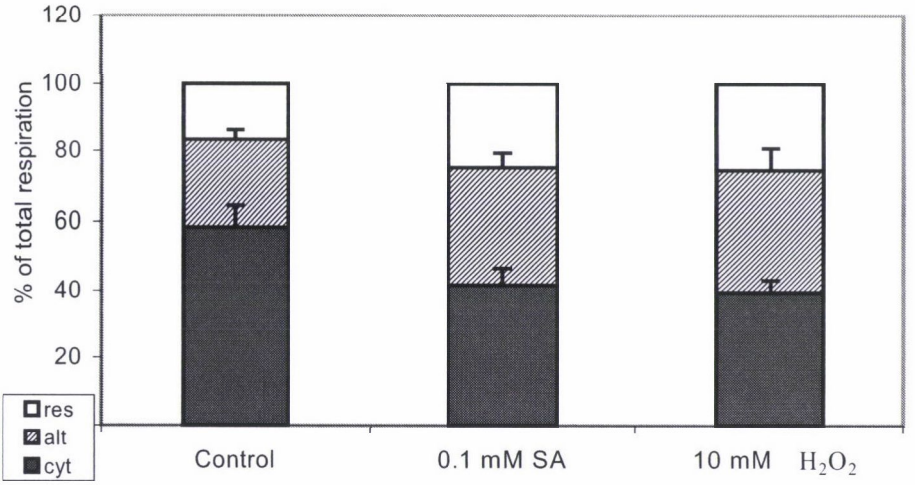

Fig. 8. Ratio of cytochrome (cyt), alternative (alt) and residual (res) respiration in maize (*Zea mays* L., hybrid Norma) roots as a percentage of total respiration in the control and after 1 day of treatment with 0.1 mM SA or 10 mM H_2O_2

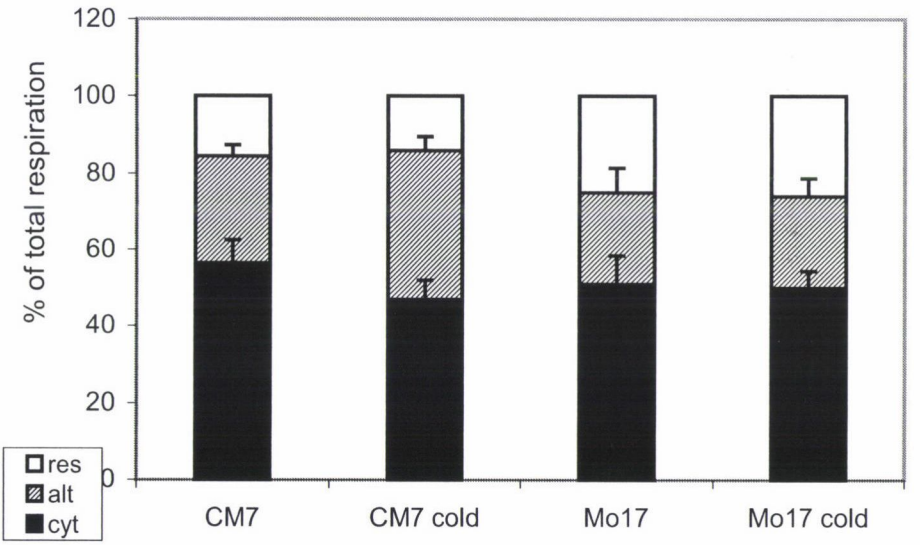

Fig. 9. Ratio of cytochrome (cyt), alternative (alt) and residual (res) respiration as a percentage of total respiration in the roots of inbred maize lines Mo17 (chilling-sensitive) and CM7 (chilling-tolerant) at control temperature (22°C) and during chilling treatment (12°C)

In addition to electrolyte leakage, various photosynthetic parameters (net CO_2 assimilation, the F_v/F_m parameter indicative of the maximum efficiency of PSII) were also tested. Although in some cases these parameters also revealed the positive effect of SMM, the differences were not statistically significant under the given experimental conditions.

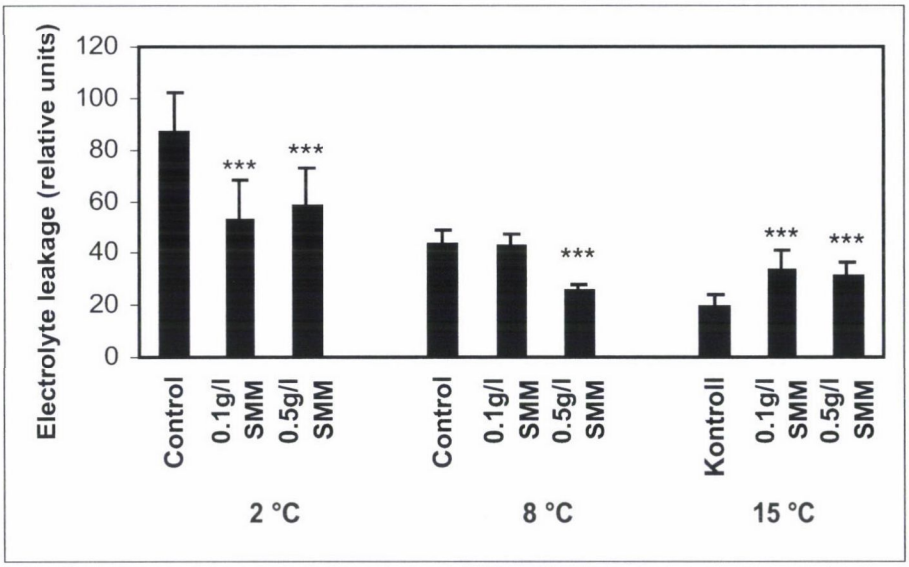

Fig. 10. Effect of S-methylmethionine treatment on the extent of electrolyte leakage induced by chilling stress in the maize line CM7 (***: Significant difference from the control at the $p \leq 0.05$ level)

Conclusions

The results achieved in the research carried out as part of the Maize Consortium Project can be summarised as follows:

a) Salicylic acid may inhibit the uptake of cadmium, but it also causes damage to the roots, including a reduction in PCS activity, so preliminary treatment with SA may aggravate the damaging effect of Cd.

b) A comparison of the chilling tolerance levels of maize genotypes and the activity of antioxidant enzymes indicates that the two parameters are not directly related to each other, so the chilling tolerance of inbred maize lines or hybrids cannot be reliably determined from antioxidant enzyme activity levels.

c) The intensity of alternative respiration in maize roots was stimulated by both SA treatment and hydrogen peroxide treatment, confirming the suggestion that the effect of SA is exerted via a rise in the H_2O_2 level. At low temperature there was an increase in the ratio of alternative respiration in chilling-tolerant maize genotypes, while no change was observed in chilling-sensitive plants. For this reason, a larger quantity of reactive oxygen species is produced in the mitochondria in the latter, possibly contributing to greater damage to the plants.

d) In the course of low temperature stress, SMM was able to reduce the level of electrolyte leakage, i.e. it led to a significant decrease in the extent of membrane destruction. Nevertheless, although there was a slight positive trend, no significant correlation was found between SMM and various photosynthetic parameters.

Acknowledgements

The authors are gratefully indebted to Zsuzsa Koti and Edit Kövesdi for their technical assistance. This work was supported by grants from the Ministry of Education (NKFP 4/008/2002) and from the Hungarian National Scientific Foundation (OTKA T37987).

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Acta Agronomica Hungarica, 53(2), pp. 197–210 (2005)

ELECTRICAL CAPACITANCE OF ROOTS IN RELATION TO PLANT ELECTRODES, MEASURING FREQUENCY AND ROOT MEDIA

K. RAJKAI[1], K. R. VÉGH[1] and T. NACSA[2]

[1]RESEARCH INSTITUTE OF SOIL SCIENCE AND AGRICULTURAL CHEMISTRY OF THE HUNGARIAN ACADEMY OF SCIENCES, BUDAPEST, HUNGARY
[2]N-TELEKOM CO. LTD., KISKUNHALAS, HUNGARY

Received: 25 February, 2005; accepted: 26 May, 2005

The electrical capacitance method was applied for the examination of living root systems in a pot experiment. The measured root capacitances gave an unambiguous indication of the development of root mass and length. The root capacitances measured using needle and clamp plant electrodes were closely similar when the roots of whole plants were placed in water, while increasing differences were observed with a decrease in soil water saturation. The difference in capacitance between the plant electrodes is outlined by interpreting the action mechanism of the clamp electrode.

The capacitance and electrical impedance spectra (30 Hz–1 MHz) were determined for roots in soil, for pieces of roots washed free of soil, and for the soil itself. The root capacitance was smaller than that of the soil and higher than that of root pieces at 1 kHz, while the capacitance of the soil became equal to that of roots in soil at about 2 kHz. This calls attention to the importance of the measuring frequency when determining root capacitance.

A capacitor model with two dielectric media is proposed besides Dalton's model in order to interpret the behaviour of root and soil capacitances. However, its validity requires further verification.

Key words: plant electrode, frequency dependence, root-soil capacitance model

Introduction

Root extension and mass are of great importance from the point of view of both plant nutrient and water uptake. The inspection of the growth and activity of the root system, however, is greatly hindered in the soil. Conventional soil coring methods of root analysis (e.g. Polomski and Kuhn, 2002) destroy roots and plants making it impossible to make continuous observations on the roots of individual plants.

The rhizotron or minirhizotron techniques facilitate the continuous observation of plant roots. However, these techniques change the soil conditions in the vicinity of the observation tubes, and consequently the root densities determined with the minirhizotron and core sampling techniques may differ (e.g. Andrén et al., 1993).

Due to the methodological difficulties encountered in root analyses, a great need exists for a simple, rapid analytical method capable of providing information on the *in situ* root status without damaging either the structure or the life functions of the plants.

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Hayden et al. (1969) studied the electrical impedance of potato and alfalfa roots and stems. Chloupek (1972) studied root size parameters, such as length, mass and characteristic diameter, and root function parameters for various cultivated plants in different soils. He used a capacitance bridge (Tesla BM394E) with a frequency of 1 kHz to polarize the root biological membranes and connected it to a needle plant electrode inserted into the suction zone of the root crown and to a metal rod inserted into the soil. The electric capacitance technique was used to select plants with greater root mass for the purposes of breeding alfalfa (Chloupek et al., 1999). He found that the measured capacitance value depended to a great extent on the water content of the soil.

Instead of using a needle, Kendall et al. (1982) used a spring tension clamp plant electrode and analysed the relationship between the root capacitance and root morphology. To study the root mass of maize genotypes, a battery clamp was used to make electrical contact with the plants 6 cm above ground level (van Beem et al., 1998). A survey of published root capacitance measurements is given in Table 1.

Table 1
Survey of published root capacitance measurements

References	Plants	Exp. layout	Root medium	Root traits	Plant electrode	Potential (V)	Frequency (kHz)
Chloupek, 1972	Maize	Pot	Quartz sand	Surface area	Needle	12	0.8
	Oat	Pot	Clay soil	Surface area	Needle	12	5
	Onion	Pot	Quartz sand	Surface area	Needle	12	1
	Rape	Pot		Surface area	Needle	12	0.8–5
	Sunflower	Pot	Quartz	Surface area	Needle	12	5
	Sunflower	Pot	Clay	Surface area	Needle	12	5
Chloupek, 1977	Carrot	Field	Loam soil	Carrot biom.	Needle		
	Mustard				Needle	0.1, 1, 10	0.1, 1, 10
	Oat				Needle	0.1, 1, 10	0.1, 1, 10
	Onion				Needle	0.1, 1, 10	0.1, 0.5
	Red clover	Field			Needle		
	Sunflower	Pot	Sand	Mass	Needle		0.8, 5
Chloupek, 1980	Alfalfa	Field	Light soil	*Root system size	Needle		1
Kendall et al. 1982	Alfalfa	Field	Silt loam	Dry m., diam.	Clamp		1
	Red clover	Pot	Hydroponics	Dry m., diam.	Clamp		1
Dalton, 1995	Tomato		Hydroponics	Mass	Needle		1
			Sand		Needle		1
Matsumoto et al. 2001	Maize	Pot	Soil	Dry m. length	Needle		N. a.
Ozier-Lafontaine et al.	Maize	Pot	Soil	Dry mass	Needle		1
2001	Carrot	Field	Soil	Dry mass	Needle		1
Van Beem et al. 1998	Maize	Pot	Vermicullite	Fresh mass	Clamp		1
	Maize	Field	.Loam	Fresh mass	Clamp		1

*Characterized by the measured electrical capacitance; biom: biomass; Dry m.: Dry mass; N. a.: Not available

On the basis of experiments and experience with root capacitance measurements, Dalton (1995) presented a parallel resistance-capacitance model for roots in soil, and demonstrated that the root mass and root length derived from electric capacitance values express the actual size of the active root surface.

In Dalton's root capacitance model the soil is a resistor and provides the contact medium between the root and the soil electrode. The soil water content determines the resistance and the size of the contact surface between roots and soil.

The first objective was to compare the usability of needle and clamp plant electrodes for measuring the root capacitance at different growth stages and in different root media. The second objective was to study the frequency dependence of root capacitance and to analyse the electrical properties of roots and soil.

Materials and methods

Growth of sunflower plants

Twelve sunflower plants were grown under controlled temperature and light conditions in 2-litre soil-filled plastic pots in the laboratory. The soil used was a sandy subsoil having a fine sand texture, 2.7% lime content, 7.62 pH value and no organic matter. Each pot was filled with 2.65 kg of air-dry soil on gravel resting on sieve tissue. Long Ashton nutrient solution was used for plant nutrition (Hewitt, 1963). During plant growth, the water content of the soil in the pots was checked daily by weighing and was maintained at around field water capacity (23–25% $cm^3\ cm^{-3}$) by irrigation with water and/or Long Ashton solution. The root capacitance of the sunflower plants was measured at three growth stages (in the 8-, 14- and 18-leaf stages). At each growth stage four sunflower plants were measured in their pots with the same electrical arrangements using needle and clamp plant electrodes. Following the root capacitance measurements in the pots, the root system of each plant was washed free of soil, then immersed in a glass flask filled with water, after which root capacitance measurements were made again as in the soil-filled pots. The sunflower roots were submerged in water, imitating their position in the soil. The distance of the plant electrodes from the soil and water surface was approximately the same.

Measuring the mass and length of sunflower roots

At each growth stage the four replicate plants were harvested after the root capacitance measurements in water. The shoots were cut off just above the root crown, then the fresh mass of the whole root system was determined by weighing. The total length of the root system was measured with a Delta-T root length and area meter fitted with a video camera (Webb, 1989).

Measuring instruments, electrodes and layouts

The electrical capacitance measurements were made using a GW814 simple portable hand instrument and a HP4284A precision lab LCR instrument. The GW814 instrument measures the capacitance up to 2 μF using a frequency signal of 1 kHz and 1 V terminal voltage. Its capacitance corresponds to that of the parallel connected capacitance and resistance. This instrument was used for measuring root capacitances at each growth stage of plants in pots and plants with washed roots standing in water.

Electrical impedance, phase shift and loss factor were recorded on one sunflower plant (at the 18-leaf stage) between 30 Hz and 1 MHz using a computer-driven HP4284A instrument, with a terminal voltage of 1 V. Details of the measuring setup can be found in Felföldi et al. (1993). The capacitance measurements on the sunflower rooted in soil and on the soil itself were made in

capillary-saturated soil having a 27.3% ($m^3\ m^{-3}$) water content, measured in the pot with a TRIME FM2 TDR soil water content meter and a P2 probe. The water content meter was calibrated according to the factory instructions.

All capacitances in this paper are given according to the parallel connected capacitance and resistance arrangement (C_p).

A hypodermic needle, and a spring tension clamp with flat ends of about 0.5 cm × 0.3 cm, were used as plant electrodes. The needle was inserted into the stem just above the root crown at an angle of about 45° as far as the suction zone. Before the clamp electrode was installed, a thin layer of electrocardiographic paste (UNIGEL) was smeared around the stem (Kendall et al., 1982) at the point where the needle was previously inserted. The clamp plant electrode was clipped onto the gel-smeared stem avoiding any contact between the electrode or gel and the soil. The physical size and geometry (rectangle) of the clamp's tip is not significant since the smeared conducting gel determines the effective surface area of the plant electrode.

For measurements on the root capacitance of sunflowers rooted in soil, the ground wire of the capacitometer was connected to the 6.3 mm diameter stainless steel probe of the Trase System1 soil water content meter (Soil Moisture Corp., 1990) inserted about 10 cm into the soil at about 6–8 cm from the stem. The same arrangement was used for roots in water. A schematic diagram of the root capacitance measuring setup is given in Figure 1.

For measurements on root pieces, the root system of one 18-leaf sunflower plant was washed out of the soil after measuring the capacitance of the plant when rooted in the soil. The washed root system was cut from the stem just above the root crown and laid on blotting paper. The impedance, capacitance and phase angle were recorded between two needle electrodes inserted 5 mm apart into a piece of root 1.5 mm in diameter at a distance of about 3 cm from the root crown. To measure soil capacitance, two stainless steel soil electrodes were pressed into the soil in the pot with a separation of about 8 cm. The water content of the soil was 27.3% ($m^3\ m^{-3}$). The wires of the HP4284A capacitometer were connected to the needle electrodes in the case of root pieces and to the soil electrodes for soil capacitance measurements.

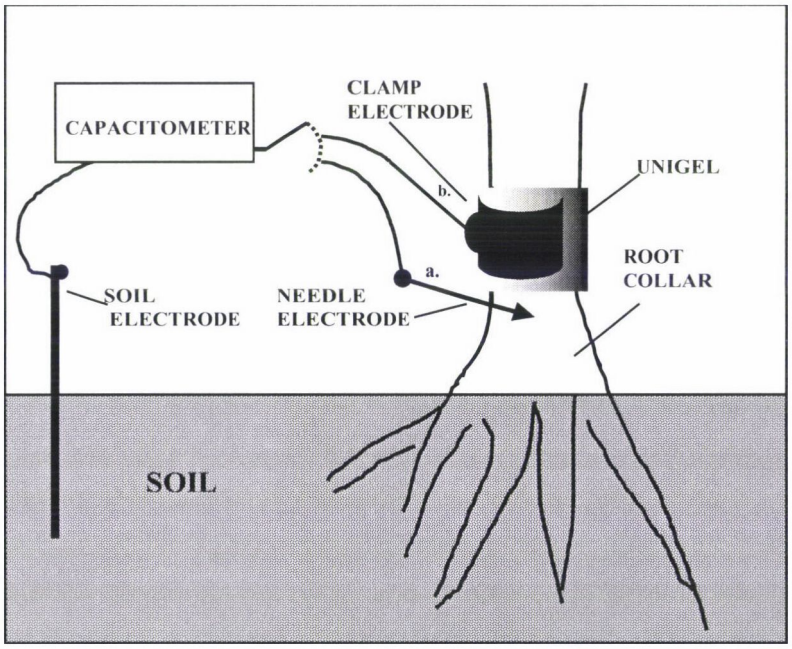

Fig. 1. Schematic view of the root capacitance measuring arrangement with needle (a) or clamp plant electrode (b) in soil root medium

Theory

Electric capacitance and its use in the root-soil system

In electrical theory, capacitance is expressed as the quantity of electrical charge accumulating on two parallel conducting plates as the result of a given electric potential (Hilhorst, 1998). The size of the charge depends not only on the size of the charge-storing surface, but also on the distance between the plates and on the permittivity of the dielectric material between them:

$$C = \varepsilon \varepsilon_0 A \cdot l^{-1} \quad (1)$$

where ε_0 is the permittivity of free space (8.85×10^{-12} F m^{-1}), ε is the relative permittivity or dielectric constant of the material, A is the area of the charge-storing surface, and l is the thickness of the dielectric medium or the distance between the capacitor plates.

The capacitances of parallel-connected capacitors are additive, whereas the reciprocal capacitance of series-connected capacitors is the sum of the reciprocals of the capacitances. The capacitance of capacitors with two dielectric media is identical with the capacitance of two series-connected capacitors (e.g. Budó, 1972).

However, in root-soil systems such ideal capacitors do not exist. Due to the applied electrical field (E-field) charge-storing surfaces may develop both in the root tissues and in the soil material and the stored charges are capable of being mobilized. Under the influence of an E-field the water molecules may align themselves with the force lines of the E-field. The process of induced-charge alignment is called polarization. To equilibrate the altered charge distribution due to polarization, the polarized and ionic charges shift, causing an electric current. This current is proportional to the extent of electric polarization and can be measured (e.g. Budó, 1972).

In order to achieve polarization in the root-soil system, a range of electrical signal frequencies was used as shown in Table 1. The most frequently used frequency is 1 kHz (Kendall et al., 1982; Dalton, 1995; Ozier-Lafontaine et al., 2001; van Beem et al., 1998; Preston et al., 2004). According to Dalton (1995) the charge developed during root capacitance measurement is the result of membrane surface polarization in the root. Dalton regarded the root wall membranes as insulators and the xylem sap and soil solution as conductors. He considered the xylem sap as the interior conductor in contact with the needle plant electrode, and the soil solution as an exterior conductor in contact with the soil electrode. The two conductors are in contact with each other at the outer root surface. Dalton interpreted the measured root capacitance as the summed value of active root elements, i.e. those in contact with the soil solution, considering them as parallel-connected cylindrical capacitors. He considered the soil water content, and the ionic composition and concentration of the soil solution to have a decisive effect on the resistance of the root capacitance circuit.

The experimental observations listed in Table 1 show that root capacitances were measured at a frequency of 1 kHz in most cases. The use of this frequency may be attributed to the permittivity change taking place in the water-air contact surfaces (Hilhorst, 1998). The hypothesis applied in Dalton's model, that the xylem and the soil solution are conductors, is supported by film polarization theory (Dukhin and Shilov, 1974).

Frequency dependence of permittivity

Permittivity is frequency-dependent (Debye, 1929). The relative permittivity (ε) of water at zero frequency at 20°C is $\approx$ 81, and it decreases with increasing frequency (Hilhorst, 1998). Because of the frequency dependence of permittivity, root capacitance was studied as a function of frequency.

Electrical impedance

Ohm's law is valid for alternating current electric circuits. The impedance and capacitance of the root-soil electrical circuit can be measured using two equivalent circuits (parallel and series combinations). The parallel combination is used for capacitive objects. In the case of a resistor–

capacitor circuit, the electrical impedance is a function of the effective resistance (R_e) and the capacitive resistance or reactance (X):

$$Z = \sqrt{R_e^2 + X^2} \tag{2}$$

where $R_e = Z \cdot \cos\varphi$, $X = Z \cdot \sin\varphi$ and φ is the phase-shift angle.

In the case of ideal capacitors, the value of φ is 90° and there is no effective energy loss. The measurable energy loss arises from the ohmic and dielectric losses. The frequency-dependent energy loss can be expressed by the loss factor (D). D is positive in the parallel combination and can be written as follows (Budó, 1972):

$$D = \frac{1}{2 \cdot \pi \cdot f \cdot C_p \cdot R_p} \tag{3}$$

where f is frequency (Hz), C_p is parallel combination capacitance (F) and R_p is parallel combination resistance (Ω).

Phase shift of the measuring frequency

The phase-shift angle used for root capacitance measurements provides information about the capacitive character of the measured root-soil object. The phase angle is –90° for ideal capacitors. No phase shift (φ=0°) is characteristic of pure ohmic resistances (Budó, 1972).

Results

Root capacitance and root mass and length

The electrical capacitances of sunflower roots in pots, measured between needle and clamp plant and soil electrodes with the GW814 capacitometer, were plotted against the mass and length of the roots, as illustrated in Figures 2 and 3. The figures show that root capacitance provides a good indication of the growth of both fresh root mass and length. It can be seen that root mass correlates better with root capacitance (R^2>0.8) than root length (R^2<0.8) when measured either with a needle or a clamp plant electrode. This may reflect the higher accuracy of root mass measurements compared to that of root length. Root capacitances measured with needle electrodes on fresh root material change linearly with root mass and length at the 4- and 8-leaf growth stages. However, the root capacitances of sunflower at the 18-leaf growth stage show high variance when measured with either a needle or a clamp electrode. The higher variance of the root capacitance at the 18-leaf stage may reflect the difference between the active and bulk root mass. The high variance in root capacitances plotted against root length started at the 8-leaf growth stage when measured with a clamp electrode (Fig. 3). However, the slopes of the linear relations between the root capacitance and root mass or length did not differ statistically.

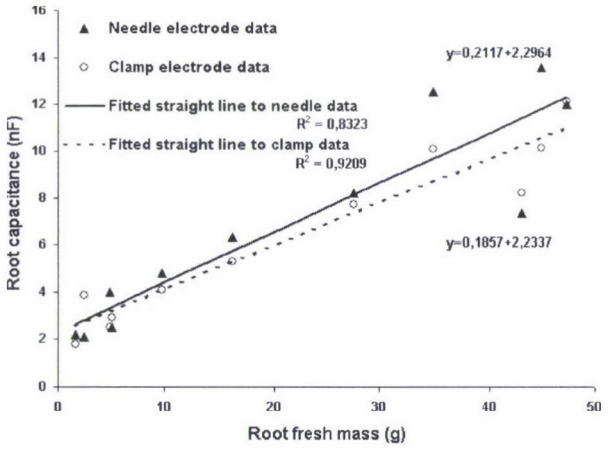

Fig. 2. Root capacitance in soil measured with needle and clamp electrodes on a GW814 instrument, plotted against the fresh root mass of sunflower plants measured at different growth stages

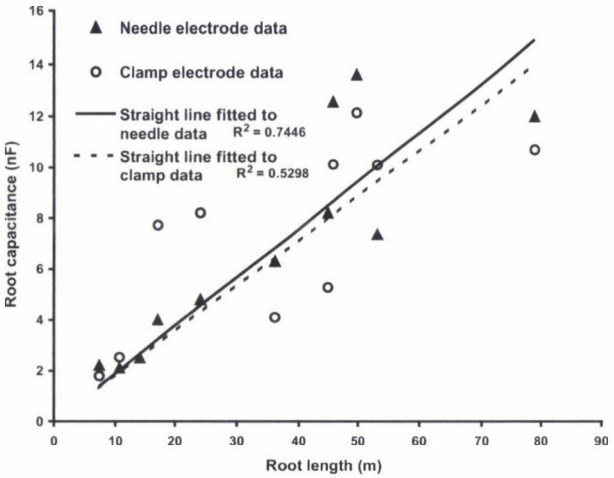

Fig. 3. Root capacitances in soil measured with needle and clamp electrodes on an GW814 instrument, plotted against the root length of sunflower plants in different growth stages

Root capacitances measured with needle and clamp plant electrodes

Root capacitances were measured using needle and clamp plant electrodes at three (4-, 8- and 18-leaf) growth stages on sunflower plants in soil field capacity (θ=23%), in capillary-saturated (θ=27.3%) and in water. The root capacitance data measured in soil at different water contents and in water are presented in Figure 4. The smallest difference between capacitances, i.e. where the slope of the fitted straight line is the closest to unity, was measured with needle and clamp plant electrodes in water. The difference in the sunflower root capacitances was greater in soil at field capacity than in capillary-saturated soil. The scattering of root capacitance data increased above 10 nF (Fig. 4). The root

capacitances measured with needle and clamp plant electrodes were the most similar in water.

The root capacitance (Fig. 5) and impedance spectra (Fig. 6) of one 18-leaf sunflower plant were measured between needle and clamp plant and soil electrodes in a pot with capillary-saturated (θ=27.3%) soil. Over the frequency range used, the values recorded with the clamp plant electrode were about 150 Ω higher for impedance (not shown) and 2 nF smaller for capacitance (Fig. 5) than with the needle electrode.

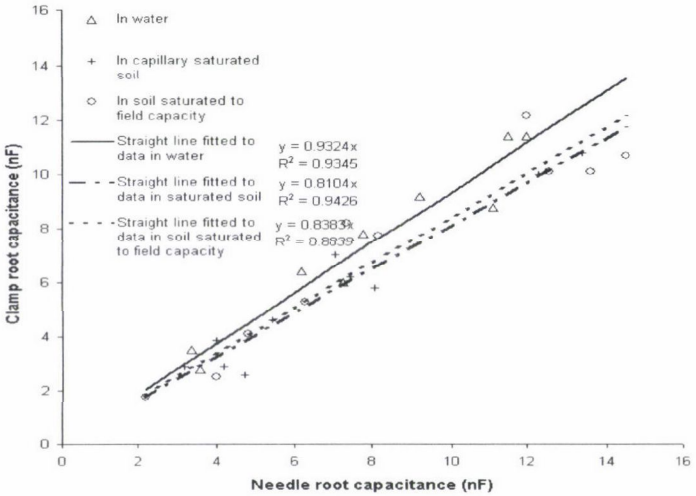

Fig. 4. Root capacitances of sunflower plants measured with needle and clamp electrodes on a GW814 instrument in water, in capillary-saturated soil and in soil at field capacity

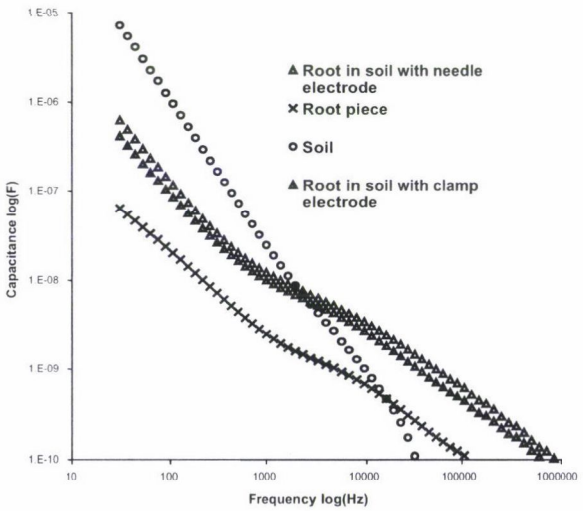

Fig. 5. Capacitance (C_p) spectra of a root piece, sunflower roots in soil, and the soil measured with the HP4284A instrument

Evaluation of the measured root capacitances and impedances

Root capacitance and impedance values measured in the 30 Hz to 1 MHz frequency range on an 18-leaf sunflower plant in capillary-saturated soil, on a piece of washed root, and in the soil are shown in Figures 5 and 6. It can be seen in Figure 5 that the capacitance spectra of the root piece and the roots in soil run almost parallel, while that of the soil is quite different. The capacitance (C_p) of the root piece, the root in soil, and the soil were 2.4, 12.2 and 16.0 nF, respectively, at 1 kHz (Fig. 5). In general the C_p of the root and the soil at 1 kHz increased towards lower frequencies and decreased towards higher frequencies to various degrees.

The electrical impedances (Z) of the root and soil measurements are given in Figure 6, and for three of the frequencies (lowest, normal and highest) used in the root capacitance measurements in Table 2. For the soil Z was almost constant over the whole frequency range, and for roots in soil it gradually decreased from 1.6 to 1.3 kΩ, while the Z value of the root piece decreased from 13.6 to 10.6 kΩ between 500 Hz and 5 kHz.

The phase angle (Φ) spectra for the roots of an 18-leaf sunflower in capillary-saturated soil, for a piece of washed root, and for the soil are given in Figure 7. Phase angle values are given for three frequencies in Table 2. If Φ is negative this indicates phase-lead, and the value of Φ indicates the strength of the capacitive character. The phase-lead angle spectrum, which shows the capacitive character of roots in the soil, is fairly similar to that of the root piece. The phase angle values show that the capacitive character of the soil is rapidly lost above 0.5 kHz, and practically disappears above 10 kHz (Fig. 7).

The effective resistance (R_e) and reactance (X) of the root-soil system, calculated from Z using Eq. (3), are given in Table 2. The reactance of the root piece is about ten times greater than that of roots in the soil.

Table 2

Electrical parameters of the root and soil in the frequency range used for root capacitance measurements

Electrical parameters	Root in soil			Root piece			Soil		
	0.5 kHz	1 kHz	5 kHz	0.5 kHz	1 kHz	5 kHz	0.5 kHz	1 kHz	5 kHz
Z (kΩ)	1.60	1.55	1.35	13.60	12.90	10.70	0.37	0.37	0.36
Φ°	–5.96	–6.84	–11.60	–11.70	–11.40	–16.60	–2.63	–1.85	–1.01
D	9.60	8.26	3.09	5.04	4.96	4.74	21.10	22.80	25.20
R_e (kΩ)	1.60	1.54	1.32	13.30	12.60	10.20	0.11	0.11	0.11
X (kΩ)	0.17	0.19	0.27	2.77	2.54	3.06	0.04	0.02	0.01

Z: impedance; Φ: phase-shift angle; D: loss factor; R_e: effective resistance; X: reactance

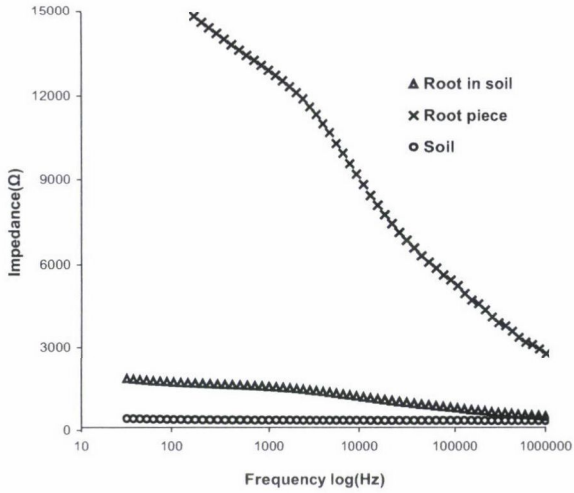

Fig. 6. Electrical impedance (Z) of sunflower roots in soil, root pieces and soil, measured with a needle electrode and the HP4284A instrument

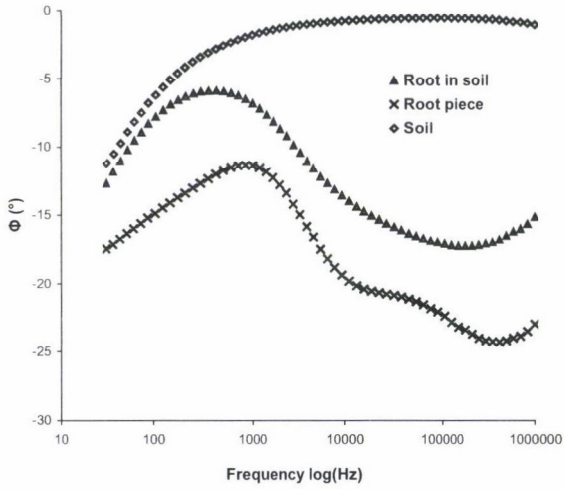

Fig. 7. Phase angle (Φ) spectra of a root piece, the root of an 18-leaf sunflower plant in soil, and the soil, measured with the HP4284A instrument

Operation of the clamp plant electrode

The evaluation of the root capacitances measured with needle and clamp plant electrodes requires an understanding of the operation of the clamp plant electrode, since, in contrast with the needle electrode, it is not in direct contact with the xylem sap. An attempt will be made to explain how to obtain similar capacitances to those recorded with the needle plant electrode, and to pinpoint possible causes of deviation.

The alternating frequency signal of the capacitometer is connected to the clamp plant and soil electrodes. The E-field of the signal conducted by the high conductivity gel around the stem penetrates into the root crown tissue, where it polarizes the membrane surface charges of the cell walls in the suction zone of the root. This polarization induces a charge shift. It is assumed that no membrane polarization takes place in the outer stem tissues, as these have no membrane surfaces with continuous water or solution film cover. The polarization energy of the E-field spreading from the clamp electrode, however, is considerably weaker than that of the needle electrode. This is demonstrated by the loss factor (D) spectra measured in sunflower roots (at the 18-leaf stage) in capillary-saturated soil with needle and clamp plant electrodes (Fig. 8). The value of D is 9.6 for the clamp and 8.3 for the needle electrode at 1 kHz, a difference of 16%, corresponding to the higher Z and lower capacitance measured with the clamp electrode (Fig. 5).

Discussion

The increased scattering of the root capacitance data of the 18-leaf sunflower plants (Figs. 2 and 3) indicates more variable root activity than could be predicted from the root mass or length. This might be because the root mass and length suffer from either a lack of root hairs or a surplus of inactive or dead roots.

The parallel combination capacitances (C_p) at different frequencies demonstrated different behaviour for roots in soil and for the soil (Fig. 5). Between 500 Hz and 5000 Hz the phase-shift angle of the soil decreased, while that of roots in soil increased substantially. This observation makes it possible to use a model of a capacitor having two dielectric media to describe the root-soil system in the frequency range where both the root and the soil have capacitive character in series connection. The elements of the two-dielectric capacitor model can be identified for the root system in soil as shown in Figure 9.

The two-dielectric capacitor consists of charge-storing conductive capacitor surfaces and two dielectric media with different permittivity. The electrode surfaces of the electrically-conductive capacitor are activated in the root and soil by the E-field of the measuring signal. Polarization starts at the plant electrode in the root and at the soil electrode in the soil. The capacitance of the two-dielectric capacitor is the product of the polarized charges accumulating on the polarized capacitor surfaces in the root and in the soil. The non-polarized root tissues and soil material form the dielectric media between the capacitor plates. The two dielectric media have different permittivity and thickness values. The contact surface area of the two dielectrics, which is not identical with the area of the capacitor plates, depends on the actual root size and activity, while the area of the capacitor plates depends on the actual properties of the root and soil.

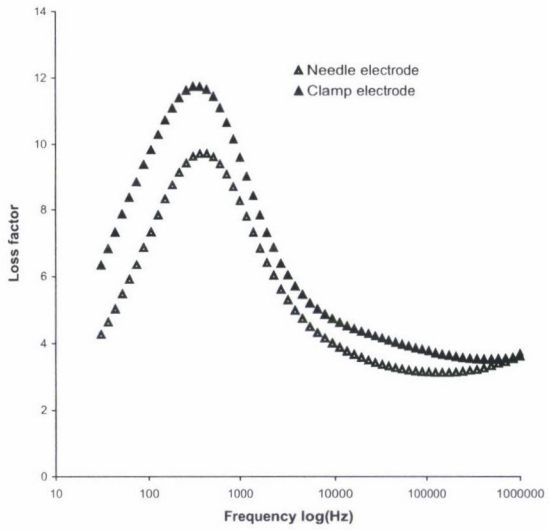

Fig. 8. Loss factor (D) spectra of sunflower roots in capillary-saturated soil, measured with needle and clamp plant electrodes with the HP4284A instrument

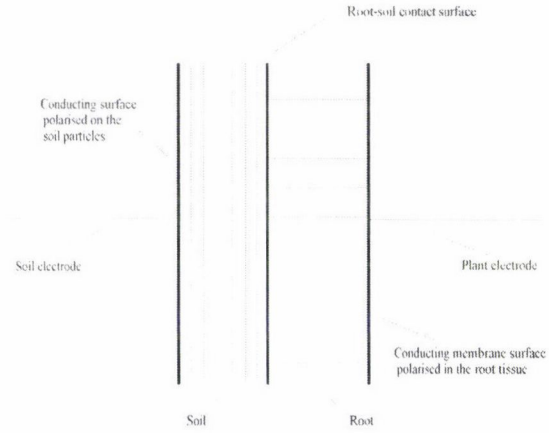

Fig. 9. Root and soil as components of a two-dielectric capacitor

The capacitance of the two-dielectric capacitor is equal to the capacitance of two series-connected capacitors (Budó, 1972). The resulting capacitance of the two-dielectric capacitor approximates to the capacitance of the smaller capacitor, which is the capacitance of the root piece, as shown in Figure 5. Of course the capacitance of the root piece does not represent the capacitance of the whole root system, but its change with frequency is comparable with that measured for plants rooted in the soil. The same is valid for the electrical impedance (Fig. 6).

The capacitance spectra measured for the root system in soil and for the soil (Fig. 5) demonstrate the constrained validity of the two-dielectric capacitor model, because the soil capacitance becomes equal to that of sunflowers rooted in soil at about 2 kHz. The loss factor (D) values in Table 2 show that the root-soil system is composed of extremely "bad", high loss factor capacitors. The highly frequency-dependent nature of the root-soil capacitor system, however, calls attention to the importance of the measurement frequency.

Since the experimental results were obtained under controlled laboratory conditions and only for sunflower plants, the applicability of the two-dielectric capacitor model will need to be checked for other plant species and environmental conditions (soil types, soil water contents and chemical compositions).

The phase angle spectra (Fig. 7) also call attention to the importance of the frequency used to measure the root capacitance; 1 kHz is a good starting value, but it is not necessarily the best (Table 1).

The root capacitances obtained with a plant electrode clipped to the stem or with one inserted into the suction zone were only closely similar when the root systems were immersed in water. The difference in the capacitances measured with needle and clamp electrodes increased as the soil water content decreased (Fig. 4). The use of a clamp plant electrode needs to be further evaluated, in spite of the positive results obtained using a battery clamp for root capacitance measurements in maize (van Beem et al., 1998).

The polarizing power of the E-field in the case of the clamp electrode may change with plant development and with soil water content. The latter probably increased the difference between the root capacitances measured with needle and clamp electrodes in soils with different water contents (Fig. 4).

Conclusions

1. Root capacitances measured with needle and clamp plant electrodes have a similar relationship to root fresh mass and length, but the variance of the capacitance is greater in the case of root length.
2. With water as the root medium, a clamp plant electrode can be used instead of a needle electrode.
3. A two-dielectric capacitor model was developed in which the root medium was not a simple resistor but a series-connected capacitor. This model allows root capacitance measurements to be interpreted from different aspects.
4. Knowledge of electrical impedance and the effect of measuring frequency is necessary for designing the optimal experimental setup for root capacitance measurements.

Acknowledgements

The root capacitance study was funded by a grant from the National Scientific Research Fund (OTKA T22087), which is gratefully acknowledged. Thanks are also due to Eszter Vozáry (Department of Physics and Control, Faculty of Food Science, Szent István University) for allowing us the use of the HP4284A measuring bridge and the stepwise frequency program. The authors are grateful to Prof. A. R. Dexter for his comments and linguistic corrections.

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Acta Agronomica Hungarica, 53(2), pp. 211–222 (2005)

MICRONUTRIENTS AND YIELD QUALITY OF PEA (*PISUM SATIVUM* L.) VARIETIES SUSCEPTIBLE TO *FUSARIUM OXYSPORUM*

E. NEMESKÉRI [1] and Z. GYŐRI [2]

[1] AGRONA LTD., DEBRECEN, HUNGARY
[2] CENTRAL LABORATORY, CENTRE FOR AGRICULTURAL SCIENCES, UNIVERSITY OF DEBRECEN, DEBRECEN, HUNGARY

Received: 15 November, 2004; accepted: 4 May, 2005

The appearance of *Fusarium* species and the micronutrient content of pea varieties were investigated in different plant development periods. Field experiments were carried out for three years on six dry pea varieties grown without irrigation on a soil infected with *Fusarium* species. Green and dry plants divided into shoots (including stems and leaves), pods and seeds were used to determine the presence of *Fusarium* species and the concentration of micro- and macroelements. In the green shoots and seeds of varieties registered as susceptible to *Fusarium oxysporum*, other species such as *F. solani* and *F. culmorum* were also detected. *F. oxysporum* and *F. solani* were found in the dry seeds not only in varieties susceptible to *F. oxysporum* but in resistant ones as well. In the case of susceptible varieties the phosphorus and nickel contents in the flowering plants decreased significantly compared to those in varieties registered as resistant to *F. oxysporum*. In later stages of plant development the K and Mo contents increased significantly in the green shoots and the accumulation of Mn and Mo in the green seeds intensified. The very low B concentration in the dry pods contributed to the internal infection of dry seeds by decreasing the stability of the cell wall. In the dry seeds of varieties susceptible to *F. oxysporum* a considerable decrease in the S content was found parallel with marked increases in the Mo and Mn contents. The sugar content of green seeds decreased significantly in peas susceptible to *F. oxysporum,* but neither the protein, nor the starch or sugar contents of the dry seeds decreased in comparison to the resistant ones.

Key words: pea, micronutrients, *Fusarium oxysporum, Fusarium solani*, quality

Introduction

Fusarium wilt causes significant damage to pea production in the temperate climatic zone. Seed treatments are available for the prevention of infection caused by *Fusarium*, but these are only effective until the emergence of the pea seedlings. The belowground plant parts come into contact with the pathogens during their development, but serious damage only occurs in the absence of resistance genes. Woloshuk and Kolattukudy (1986) have demonstrated that the conidia of aggressive strains of *Fusarium solani* produce more cutinase than non-aggressive strains. In collaboration with other enzymes that decompose cell walls, cutinase enzymes penetrate through the outer tissue of the cell wall and thus promote the penetration of fungus spores (Oeser and Yoder, 1994; Vogeli-Lange et al., 1995). Factors decreasing root growth, such as soil compaction and excessive temperatures, will increase the severity of

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Fusarium root rot. This will occur even if the virulent inoculum of *F. solani* f. sp. *pisi* can only be found at a depth of 60 cm in the soil profile. According to Kraft (1996) this will result in the infection of the pea root system in the upper 20 cm of the soil profile. Tu (1994) came to the same conclusion in the course of experiments involving a gradual increase in the soil temperature from 10°C to 30°C. *Fusarium* root rot was the lowest when pea plants were grown at a soil moisture content of 75% of field capacity (Wfc). When the soil had lower (Wfc 25% and 50%) or higher (Wfc 100 %) water supplies the severity of root rot increased. The toxins produced by *Fusarium* sp. cause the inhibition of plant growth and the develeopment of tissue necroses, finally resulting in the wilting of the plants. Puskás et al. (2002) reported that though the infection of wheat grains with *F. proliferatum* did not result in external symptoms, many fungi could be found inside the grain and mycotoxins were produced. Mycotoxins, the secondary products of the metabolism, are harmful to the health of both animals and humans. A study on the toxins produced by *Fusarium oxysporum* demonstrated that they increased the spontaneous activity and aggressive behaviour of rats. The aggressive behaviour was attributed to the presence of the fusaric acid produced by *Fusarium oxysporum,* and was considered to be similar to what was experienced after the inhibition of dopamine β-hydroxylase (Snigdha et al., 1996).

The basic precondition for an improvement in life quality is that food should not contain substances harmful to the organism if consumed regularly over a long period of time. The availability of soil nutrients varies as a result of external conditions (temperature, water) and the interactions between them. Kevresan et al. (2001) claimed that in the presence of heavy metals the activity of glutamin synthetase (GS) decreased. The greatest decrease in GS activity was observed in the presence of cadmium (Cd), followed by lead (Pb) and nickel (Ni), while molybdenum (Mo) had the smallest negative effect on its activity, though excessive amounts of Mo are most frequently observed to inhibit plant growth (Kevresan et al., 2001). Cadmium accumulates in the above-ground parts of plants, but the presence of zinc checks the accumulation of this element (Badora, 2002). As zinc (Zn) binding to the proteins can produce a metal-protein compound, its physiological effect is similar to that of magnesium and manganese. Zn is able to form complexes with organic acids such as malic and succinic acid in the roots. These complexes then release the organic acids and the Zn ions are retained in the vacuole. According to studies by Doncheva et al. (2001) large amounts of Zn in the roots of pea seedlings are not toxic, since in the presence of succinate they can form metal-succinate complexes. Stoyanova and Doncheva (2002) investigated the influence of succinate treatment on zinc toxicity using plant growth and mineral uptake as stress indicators. They found that cumulative Zn supplies resulted in a decrease in the concentration of the elements Ca, Mg and P in pea roots and in an increase in the levels of Ca and N in the leaves and stem, while Zn accumulation was observed in all the plant

organs. Most of the Zn taken up was retained in the roots after succinate treatment. The lower Zn translocation to the leaves and stems seemed to be the result of zinc complexing by organic anions in the roots (Stoyanova and Doncheva, 2002). Zn inhibited the activity of the nuclease released by *F. solani* sp. *pisi* from the endocarp tissues of pea pods, even when Mn, Ca and Mg cations, which stimulate enzymes, were present (Gerthold et al., 1993).

The external factors inhibiting the proliferation of *Fusarium* sp. *pisi* do not result in the death of the plants in all cases; this depends on the immune response of the varieties. The aim of this study was to investigate the occurrence of *Fusarium* species and micronutrients in different developmental stages of pea plants and to demonstrate the yield quality of varieties susceptible or resistant to *Fusarium oxysporum.*

Materials and methods

From 1999 to 2001, six dry pea varieties were planted in small-plot field experiments with two random replications without irrigation. The soil of the experiments had a neutral pH (7.38), a humus content of 1.8% and a $CaCO_3$ content of 4.8%. The composition of the experimental soil sample was as follows: P_2O_5 406 ppm, K_2O 313 ppm, Mg 212 ppm, SO_4-S 5.9 ppm, Mn 22.0 ppm, Cu 11.9 ppm and Zn 2.6 ppm. The pea cultivars investigated were *Izabella, Bohatyr*, *Delta* and *Y228* with yellow seeds, and *Tyrkys* and *Luzsányi* with green seeds. Each year ten plants were sampled from each repetition at the flowering, green seed ripening and dry seed maturity stages. The green and dry plants were divided into shoots (leaves and stem), pods and seeds in order to determine their element contents. *Fusarium* species were identified from various green and dry parts of pea plants on Papavisas culture medium.

The determination of the elements was made using ICP equipment according to the method of Kovács et al. (1996, 1998) in the Central Laboratory of Debrecen University. Green and dry seed proteins were analysed using the Kjel-Foss method (MSZ 6860-4: 1981 Hungarian standard) and expressed in terms of dry matter. The total sugar content was determined with the Luff-Schoorl method (MSZ 6830-26: 1987 Hungarian standard), where 2.5 g of milled seed sample was extracted with 40% ethanol followed by protein clearing. The quantity of starch was calculated from the same amount of sample after hydrolysis with 0.31 M HCl and protein clearing. The optical density of the supernatant was tested with a polarimeter, and the amount of starch was calculated from this figure.

The temperature and precipitation during the development of the pea plants were recorded. The cumulative heat units (HU °C) were calculated using the following formula at each stage of development: $[(T_{max}+T_{min})/2] - 4$ °C, where T_{max} was the daily maximum temperature and T_{min} the daily minimum temperature. The results were appraised using the SPSS statistical program for ANOVA.

Results

The experimental soil was infected by *Fusarium* species in the following percentages: *F. oxysporum* (29%), *F. moniliforme* (21%), *F. graminearum* (15.5%) and other *Fusarium* species (34.5%). The weather in 1999 was the warmest (1184.0 HU °C) and that of 2001 the coolest (941.5 HU °C) from the sowing to harvesting of peas. These two years had a similar quantity of precipitation during this period (167.9 and 209.7 mm, respectively), while 2000

was very dry (65.4 mm). In 1999 there was a very large heat unit sum (263.5°C) and precipitation that was moderate for flowering plants (53.1 mm). These climatic conditions and soil infection with *Fusarium* together resulted in the proliferation of the pathogens in the green plants, while this was not the case in 2000 and 2001 (Table 1).

Besides *Fusarium oxysporum* and *F. solani,* smaller quantity of spores of *F. culmorum* and *F. clamydosporum* were detected in green shoots than in dry shoots in 1999. In flowering plants of the varieties *Izabella* and *Delta*, registered as resistant to *F. oxysporum,* the spores of *Fusarium oxysporum* and *F. solani* appeared. Both *F. solani* and *F. culmorum* were detected in the green pods and green seeds of pea varieties susceptible to *F. oxysporum* (Table 1). In the years 2000 and 2001 neither the drought nor the large amounts of precipitation were favourable for the proliferation of *Fusarium* species in the green plant parts from flowering to green seed ripening. In these years it was primarily from dry shoots that a large number of spores of *F. oxysporum* and *F. solani* were isolated. The results of tests on dry seeds were somewhat contradictory. In some years certain *Fusarium* pathogens appeared in dry seeds of both resistant and susceptible varieties.

Table 1

Fusarium species (spores per 1 g matter) in different parts of pea varieties

Parts	Variety	1999				2000	2001	
		F. oxy.	F. sol.	F. culm.	F. chl.	F. oxy.	F. oxy.	F. sol.
Flowering plants	Izabella[+]	262.5	225.0	–	–			
	Delta[+]	1107.5	2760.5	–	–			
	Y228	25.0	–	–	–			
	Bohatyr	–	25.0	–	–			
	Tyrkyz	12.5	–	–	–			
	Luzsányi[+]	–	–	–	–			
Green shoots	Y228	–	58.5					
	Bohatyr	–	1411.5	319.0	–			
	Tyrkyz	–	92.0	–	–			
Green seeds	Tyrkyz	–	125.0	–	–			
Dry shoots	Izabella[+]	–	5044.0	–	719.0		100000	–
	Delta[+]	–	4994.0	–	–	2200	60000	–
	Y228	15.5	182.0	–	–	–	24900	175250
	Bohatyr	194.0	1989.0	388.5	2248.0	1025	–	–
	Tyrkyz	130.0	210.0	–	–	2050	325000	–
	Luzsányi[+]	–	189.5	–	13.5	–	6525000	25000
Dry seeds	Izebella[+]	–	–	–	–		25000	–
	Delta[+]	–	–	–	–	–	–	–
	Y228	25.0	–	–	–	–	–	–
	Bohatyr	–	–	–	–	–	–	–
	Tyrkyz	–	–	–	–	25	–	–
	Luzsányi[+]	–	12.5	–	–	–		–

F. oxy. = *Fusarium oxysporum*, F. sol. = *F. solani*, F. culm. = *F. culmorum*, F. chl. = *F. chlamydosporum*; [+]Varieties registered as resistant to *F. oxysporum*

Studies were also made on differences between element contents in flowering plants, green pods, green seeded plants and dry plants of groups susceptible and resistant to *Fusarium oxysporum*. The findings revealed that in flowering plants, the phosphorus and nickel contents decreased significantly in the *Fusarium*-susceptible group compared with the resistant one. The differences in Ni content between the two groups were large, particularly in dry years. Comparing the dry and wet years the Ni content in the green pod stages differed from that in the flowering stage. However, there were no differences in the Ni contents of the green shoots and pods in the *Fusarium*-susceptible group, though in dry years the Ni levels were significantly higher in the green seeds than in those of the resistant group (Fig. 1).

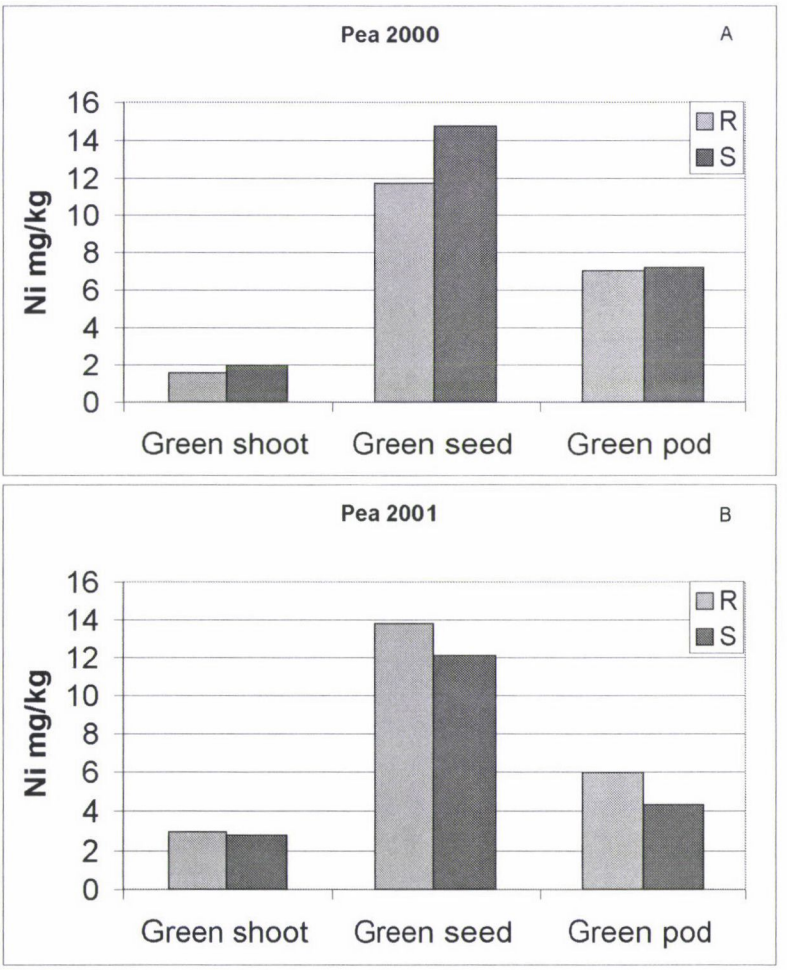

Fig. 1. Nickel (Ni) content in the organs of peas with resistance (R) or susceptibility (S) to *Fusarium oxysporum* (A) in a very dry year 2000, and (B) in a moderately wet year, 2001. The differences between the R and S groups were not significant.

The element contents of pea plants differed between the two groups at green seed maturity, as seen in Table 2. According to the results the potassium and molybdenum contents increased significantly in the green shoot, i.e. the stem and leaves, in *Fusarium*-susceptible varieties compared with resistant ones. No differences could be shown between the groups and years for the Cu and Mn contents of green shoots (Table 2). The Mg content of green pods in varieties susceptible to *Fusarium oxysporum* was smaller (1913.17 mg/kg) than in resistant ones (2058.90 mg/kg), averaged over the years, but significant differences between the two groups were only observed in cool, rainy weather.

There was little deviation between the groups in the micronutrient contents in green seeds of pea. There was a slight increase in the Mn and Mo levels of green seeds in peas susceptible to *Fusarium* compared to the resistant ones, but significant differences between the two groups were found only under cool, rainy weather conditions (Table 3).

Table 2

Element contents (mg/kg) in the green shoots of peas with resistance (R) or susceptibility (S) to *Fusarium oxysporum*

Element	Group	1999	2000	2001	Average
B	R	23.02 a	7.26 c	15.21 b	15.16
	S	23.23 a	5.60 c	18.83 d *	15.89
Ba	R	6.49 b	11.80 a	6.47 b	8.25
	S	6.87 b	12.38 a	6.62 b	8.62
Ca	R	11417.67 b	15023.17 a	17116.13 a	14518.99
	S	11524.00 b	14921.67 a	18339.33 a	14928.33
Cu	R	22.24	24.57	31.96	26.26
	S	21.43	35.76	35.88	31.02
Fe	R	153.30 b	194.67 b	151.95 b	166.64
	S	253.06 a	178.17 b	162.63 b	197.95
K	R	17379.17 b	19507.17 a	13745.25 c	16877.20
	S	21916.50 a ***	20131.83 a	18165.33 b ***	20071.22
Mg	R	1383.83 b	2142.00 a	2098.38 a	1874.74
	S	1373.17 b	2124.33 a	2164.50 a	1887.33
Mn	R	35.98	40.37	35.99	37.45
	S	34.94	38.97	37.82	37.24
Mo	R	2.32 b	0.42 e	0.89 c	1.21
	S	2.49 a ***	0.59 d ***	0.98 c	1.35
P	R	2216.67 a	1896.50 b	1839.63 b	1984.27
	S	2080.17 a	2038.83 b	1824.00 b	1981.00
S	R	1223.67 b	2420.17 a	2198.50 a	1947.38
	S	1116.67 b	2303.17 a	2200.33 a	1873.39
Sr	R	51.41 b	90.50 a	76.85 a	72.92
	S	53.67 b	92.90 a	85.15 a	77.24
Zn	R	16.10 a	18.88 a	9.47 b	14.82
	S	14.24 ab	19.73 a	8.18 b	14.05

Means within a row followed by the same letter are not significantly different to each other at the 5% level using Duncan's multiple range test; *,*** Significant at the $P<0.1$ and $P<0.001$ levels, respectively

Table 3
Element contents (mg/kg) in the green seeds of peas with resistance (R) or susceptibility (S) to *Fusarium oxysporum*

Element	Group	1999	2000	2001	Average
B	R	13.84 a	0.00 c	6.24 b	10.04
	S	13.15 a	0.00 c	6.57 b	9.86
Ba	R	0.88 a	0.99 a	0.00 b	0.62
	S	0.76 a	1.01 a	0.24 b	0.67
Ca	R	739.67 bc	649.17 c	864.38 a	751.07
	S	711.67 b	610.33 c	887.14 a	736.39
Cu	R	15.79 a	7.82 b	0.00 c	7.87
	S	14.93 a	8.11 b	0.00 c	7.68
Fe	R	66.47	51.18	59.84	59.16
	S	59.00	56.80	56.87	57.56
K	R	12516.67 a	10507.33 c	11814.13 b	11612.71
	S	11696.00 ab	10686.17 c	11909.33 b	11430.50
Mg	R	1180.33 b	1213.67 ab	1277.75 a	1223.92
	S	1180.83 b	1238.83 ab	1327.83 a	1249.16
Mn	R	11.92 b	9.85 c	12.43 b	11.40
	S	12.55 b	10.27 c	14.25 a ***	12.36
Mo	R	3.56 b	1.32 c	0.42 d	1.77
	S	3.87 a ***	1.28 c	0.47 d	1.87
P	R	5349.83	5014.50	5119.13	5161.15
	S	5183.67	4852.50	5365.83	5134.00
S	R	1464.00 b	2089.83 a	2098.38 a	1884.07
	S	1454.33 b	1946.33 a	2125.83 a	1842.16
Sr	R	3.06 b	9.14 b	4.02 a	3.41
	S	2.86 b	2.93 b	4.09 a	3.29
Zn	R	44.43	42.97	45.19	44.20
	S	41.44	45.27	45.97	44.23

Means within a row followed by the same letter are not significantly different to each other at the 5% level using Duncan's multiple range test; *** Significant at the $P< 0.001$ level

As seen in Tables 2 and 3, the uptake of elements and their transport to green plant parts depended on the climatic conditions. Marginal weather such as drought and excessive precipitation had positive effects on the contents of Mg, Ca, S and Sr in green shoots but hampered the uptake of P. Under similar conditions the accumulation of S in green pods and green seeds increased (Table 3).

The results showed an interesting picture as regards S accumulation in the green and dry parts of the plants. In the green pods and seeds of varieties susceptible to *Fusarium* the sulphur content was smaller than in resistant ones, but the differences in S contents between the groups were only significant in the dry shoots and seeds (Table 4). The boron concentration decreased significantly in the dry pods of susceptible varieties in comparison to resistant ones (Fig. 2).

Table 4
Element contents (mg/kg) in the dry seeds of peas with resistance (R) or susceptibility (S) to *Fusarium oxysporum*

Element	Group	1999	2000	2001	Average
B	R	10.20 a	0.00 c	3.90 b	4.70
	S	10.81 b ***	0.00 c	3.67 b	4.83
Ba	R	0.86 b	1.19 a	0.00 c	0.68
	S	0.83 b	1.16 a	0.00 c	0.66
Ca	R	969.33	940.67	881.25	930.42
	S	944.50	863.50	732.83	846.94
Cu	R	9.45 a	7.62 c	9.44 ab	8.84
	S	10.11 a	7.69 c	8.87 b	8.89
Fe	R	46.71 b	45.15 b	53.76 a	48.54
	S	55.15 a	52.65 ab	48.58 a	52.13
K	R	9885.33 b	8838.50 c	9779.38 b	9501.07
	S	10670.67 a ***	8958.00 c	9663.50 b	9764.06
Mg	R	1082.33	1162.67	1154.88	1133.29
	S	1176.17	1187.00	1120.67	1161.28
Mn	R	10.21 b	9.82 b	10.11 b	10.05
	S	11.62 a ***	10.97 a ***	11.67 a ***	11.42
Mo	R	3.05 b	1.20 c	0.43 d	1.56
	S	3.59 a ***	1.11 c	0.44 d	1.71
Ni	R		1.48	1.34	1.41
	S		1.92	1.50	1.71
P	R	5262.00 b	4413.00 b	4533.50 b	4736.17
	S	6480.00 a ***	4266.67 b	4522.00 b	5089.56
S	R	1525.83 c	2367.67 a	1908.38 b	1933.96
	S	1557.83 c	2172.00 b ***	1809.83 b	1846.55
Sr	R	4.13	4.54	4.35	4.34
	S	3.89	4.13	3.53	3.85
Zn		34.06 b	33.62 b	40.66 a	36.11
		36.37 b	35.08 b	41.72 a	37.72

Means within a row followed by the same letter are not significantly different to each other at the 5% level using Duncan's multiple range test; *** Significant at the $P < 0.001$ level

The sugar content in green seeds decreased significantly in varieties with susceptibility to *Fusarium* compared to the resistant ones, but the protein contents did not change. There were no differences between the two groups in the protein, sugar and starch contents in dry seeds, but the amount of dry matter declined significantly (Table 5). Although the pea varieties can be grown as both food and feed, the production of *Fusarium*-susceptible varieties poses a risk as canning quality may deteriorate.

Discussion

As a result of stress, especially that caused by bacterial and fungal infections, the activities of peroxidase, catalase and cytochrome oxidase increased. The presence of micronutrients is important for the functioning of

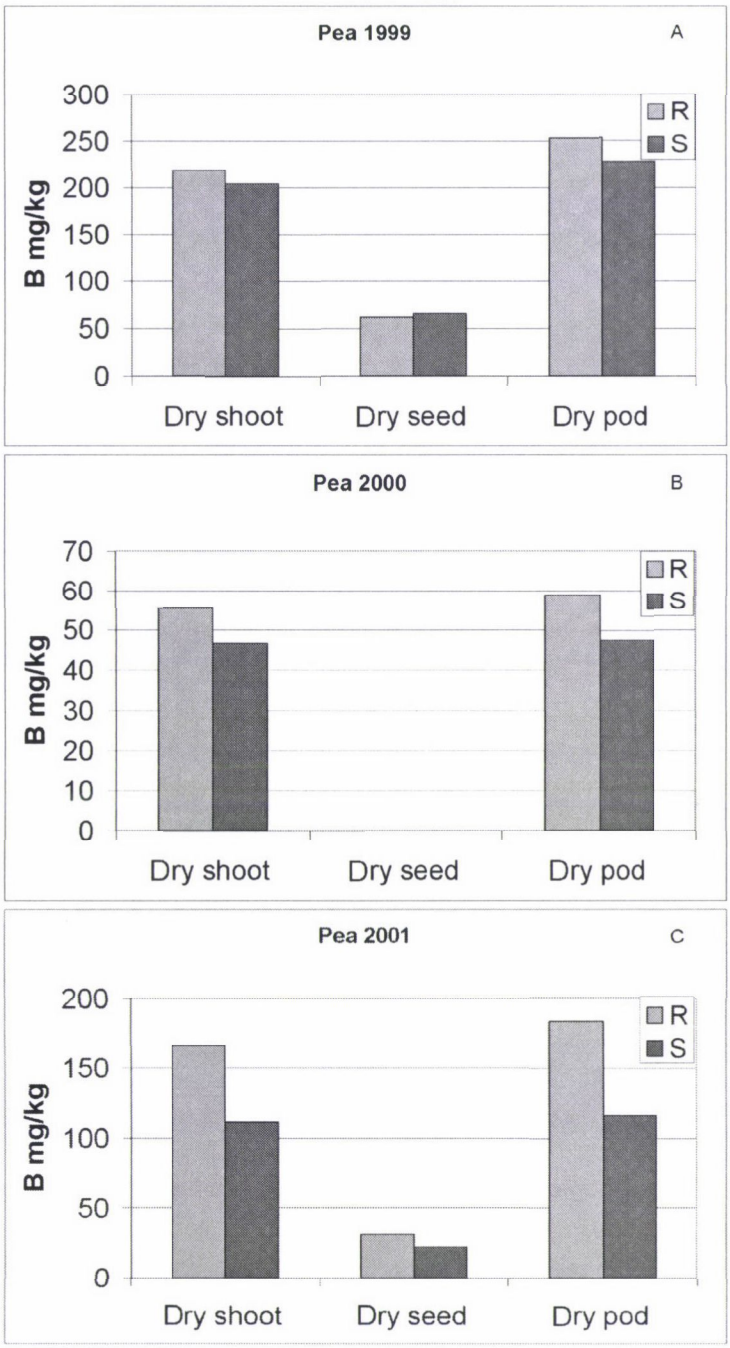

Fig. 2. Boron (B) content in the organs of peas with resistance (R) or susceptibility (S) to *Fusarium oxysporum* (A) in a wet year, 1999, (B) in a very dry year, 2000, and (C) in a moderately wet year, 2001. The difference in the B content of dry pods in the R and S groups was significant ($P< 0.05$) in years (A) and (B)

Table 5
Nutritive quality of green and dry seeds of peas with resistance (R) or susceptibility (S) to *Fusarium oxysporum* (1999–2001)

Quality	Group	Green seeds				Dry seeds			
		1999	2000	2001	Average	1999	2000	2001	Average
Dry matter %	R	26.49a	27.02ab	25.83ab	26.45	93.37a	90.77b	89.26bc	91.13
	S	28.96a	24.47b	24.57b	26.00	91.90a	88.58c	89.13bc	89.87*
Protein %	R	24.50	26.01	25.31	25.27	21.02b	25.31a	21.66b	22.66
	S	25.06	26.26	25.64	25.65	22.49b	24.73a	21.27b	22.83
Sugar %	R	8.46b	17.40a	7.29c	11.05	5.81	5.57	5.11	5.50
	S	8.93b	16.77a	5.65d	10.45***	5.98	5.77	4.94	5.56
Starch %	R	–	–	42.71	42.71	42.92	40.39	41.57	41.63
	S	–	–	43.23	43.23	42.62	41.52	41.06	41.73

Means within a row followed by the same letter are not significantly different to each other at the 5% level using Duncan's multiple range test; *,*** Significant at the P<0.1 and P<0.001 levels, respectively

these enzymes (Hornok, 1999). The decrease in nickel content was significant in the *Fusarium*-susceptible groups. It is a well-known fact that in the absence of Ni^{2+} the activity of the urease enzyme is inhibited, leading to plant death as the result of carbamide accumulation in the tissues. It is probable that the combination of *F. oxysporum* or *F. solani* and drought stress causes more significant damage to flowering plants in the *Fusarium*-susceptible group than in the resistant one. However, Ni levels increased considerably in the green seeds of the susceptible group in dry years in comparison to the resistant group.

High levels of some micronutrients result in an increase in enzyme activity. Nitrate reductase, xanthine dehydrogenase and aldehyde oxidase are plant enzymes whose activity requires the presence of molybdenum. Kevresan et al. (2001) found that the nitrate reductase activity increased at high levels of molybdenum, while the dry matter of the roots and aboveground parts decreased in the presence of molybdenum by 50 and 35%, respectively. In most cases excessive amounts of Mo resulted in decreased plant growth. According to the present results, there was a significant increase in the potassium and molybdenum content of the green shoot in *Fusarium*-susceptible varieties, compared with resistant ones. Bacterial and fungal infections contributed to intensive enzyme activity with a high K and Mo demand. The results suggest that changes in micronutrient contents in the green parts of pea are related to the plant's immune reactions to *Fusarium* pathogens.

Carpena et al. (2000) reported that a high supply of Ca induced B mobilisation from the root to the shoot. They demonstrated that the total B concentration in the cell wall of pea nodules was four times that in the roots. By contrast, the Ca content in the root cell wall was 2.5 times higher than the level found in the nodules. The results showed that in very dry years such as 2000 boron uptake by the green plant parts and green seeds in both groups was

significantly reduced (Tables 2 and 3). At high temperatures, like those experienced in 1999 and 2000, the Mn content in the pea pods was high. This finding coincided with that of Grzesiak et al. (1996), who found that the Mn content increased in plants exposed to heat stress. In rainy weather the accumulation of Cu, Zn and Mn in green pods decreased significantly.

Sulphur, in the form of the sulphydryl (SH) group, is found in all enzymes. Glutathione comprises 90% of compounds with an SH group, and due to its strong antioxidant effect contributes to the neutralisation of hydrogen peroxide. The sulphur content in the green pods and seeds of the *Fusarium*-susceptible group decreased slightly compared to the resistant one, but this decrease was significant in the dry seeds. Further investigations are needed to reveal whether low sulphur content is connected to a decrease in the quantity of antioxidant compounds or in that of S-amino acids such as methionine and cysteine.

Boron takes part in the maintenance of the cell wall structure. The boron concentration decreased significantly in the dry pods of susceptible varieties. This appears to be related to the thickness and structure of the cell wall. Thus, the low boron level appears to be responsible for the fact that aggressive fungi can penetrate the cell wall to infect the grain. The low concentrations of sulphur and boron may explain the occurrence of large amounts of spores of various *Fusarium* species in the dry shoots, as shown in Table 1.

Summing up, *Fusarium solani* can be detected in the green leaves, the stem and the seeds of pea varieties registered as susceptible to *Fusarium oxysporum* under both warm and rainy weather conditions. There were years when *F. oxysporum* and *F. solani* were isolated from the dry seeds of both susceptible and resistant varieties. The Ni content of the flowering pea plant decreased, but it increased considerably in the green seeds of the susceptible group in dry years in comparison to the resistant group. High contents of K and Mo were found in the green shoots of susceptible pea varieties. The manganese and molybdenum levels increased in green seeds to a similar extent. The low B concentration found in the pod contributed to the infection of seeds in the susceptible group by decreasing the stability of the cell wall in the dry pods.

The nutritive quality of dry seeds was very similar in both groups. The susceptible group only showed a significant decrease in the sugar content of the green seeds compared to the resistant group. There were no differences between the groups in the protein and starch contents of the dry seeds, but the micronutrient composition of the dry seeds was more favourable in the *Fusarium*-susceptible group because the Mo and Mn concentrations were higher than in the resistant group.

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Acta Agronomica Hungarica, 53(2), pp. 223–228 (2005)

DIFFERENTIAL RESPONSES OF CHICKPEA (*CICER ARIETINUM*) CULTIVARS TO IRON DEFICIENCY

Z. A. SALAMA[1] and M. M. EL-FOULY[2]

[1]PLANT BIOCHEMISTRY DEPARTMENT AND [2]FERTILIZATION TECHNOLOGY DEPARTMENT, NATIONAL RESEARCH CENTRE, DOKKI, CAIRO, EGYPT

Received: 27 December, 2003; accepted: 12 April, 2005

Most dicotyledonous species respond to Fe deficiency by developing defence mechanisms. To study the regulation of these responses a water culture experiment was conducted with four cultivars of chickpea (*ILC* 385, *ILC* 8530, *ILC* 195 and *ILC* 8522). Changes in the pH of the nutrient solution and the Fe reduction capacity were monitored over a period of 12 days to assess the efficiency of Fe uptake and utilization. The results revealed that the absence of Fe reduced the dry weight of all the cultivars. *ILC* 385 and *ILC* 8522 released more H^+ ions from the roots and reduced the pH of the nutrient solution to below pH 4.0. When the reduction of ferricyanide by intact roots was measured, an increase in the activity of NADH-Fe-CN-reductase was noted in cultivars *ILC* 385 and *ILC* 8522, while *ILC* 8530 and *ILC* 195 had limited ability to reduce pH value and Fe reduction capacity.

It could be concluded that *ILC* 385 and *ILC* 8522 seem to be more tolerant to Fe deficiency. Furthermore, this study suggests that changes in the Fe-reductase activity and in the pH of the nutrient solution may be used as laboratory techniques to assess the efficiency of cultivars in overcoming Fe deficiency stress.

Key words: Fe deficiency, proton release, Fe reduction, chickpea cultivars

Introduction

Iron deficiency is a recurring problem in chickpeas grown on high pH soils. Chickpea cultivars differ in their sensitivity to iron deficiency. It was reported by Saxena et al. (1990; 1994) that both plant species and genotypes within a given species may differ markedly in their susceptibility to Fe deficiency. It is possible to control Fe deficiency by identifying Fe-efficient genotypes that are able to acidify the root medium by increasing ATP-ase activity and Fe reduction capacity (Rayan et al., 2001).

Recently, considerable progress has been made in predicting the Fe uptake and utilization efficiency of different plant species based on their physiological and biochemical response (Dell'Orto et al., 2000; González-Vallejo et al., 2000).

Increased acidification of the rhizosphere and Fe reduction capacity are acknowledged as mechanisms through which dicots respond to Fe deficiency. It has been proposed that this process involves the activation of root plasma membrane (PM) H^+-ATPase. By pumping protons outside the cell, this enzyme contributes the proton motive force for ion uptake. In addition, its activity helps to maintain an adequate environment for Fe reductase activity (Babalakova and Daniela, 2001).

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The present study was undertaken to cast some light on biochemical mechanisms such as proton release and Fe reduction as laboratory techniques for predicting the efficiency of chickpea genotypes to overcome Fe deficiency stress.

Materials and methods

Seeds of four cultivars of chickpea (*Cicer arietinum*), namely *ILC*385, *ILC*8530, *ILC*195 and *ILC*8522 obtained from Icarda, were used. The seeds were soaked in aerated tap water and were germinated in artificial granules (Lecaton^{R}) for 3 days in the dark at 28°C. The seedlings were grown in a hydroponic culture (Hoagland and Arnon, 1950) either with or without 40 µmol iron as ferric cyanide. The hexacyanoferrate III reductase activity in intact roots was determined every 2 days for 12 days. The reduction of hexacyanoferrate III was measured spectrophotometrically using an assay solution containing 0.50 mM $K_3[Fe(CN)_6]$ substrate and 10 mM $CaCl_2$ in a pH 6.5 solution, according to Schmidt and Janiesch (1991). Reductase activity was expressed as µmol reduced Fe_4 $[Fe(CN)_6]_3$ per g root fresh weight per hour. The data presented are the means of 3 replicates for each variant. Changes in the pH of the bulk medium were measured during the reduction of hexacyanoferrate III by intact roots.

Results and discussion

Growth

The seedling growth rate, estimated from changes in dry weight during the 12-day growth period with either sufficient or deficient levels of Fe, was used as a preliminary evaluation for the response to Fe deficiency (Fig. 1).

The results indicated that all cultivars produced much less dry weight in Fe-deficient medium. *ILC* 385 was the cultivar least affected by Fe deficiency and *ILC* 8530 the most affected. Between these extremes, the cultivar *ILC* 8522 was located near the highly affected cultivar and *ILC* 195 was located near *ILC* 385. This preliminary evaluation procedure was developed to reliably distinguish chickpea cultivars according to their ability to tolerate Fe deficiency (Saxena et al., 1990).

Proton release

The changes in the pH of the nutrient solution caused by the roots of different chickpea cultivars in sufficient and deficient Fe treatments are presented in Figure 2.

Enhanced net proton release was found in *ILC* 385, *ILC* 195 and *ILC* 8522 as a response to Fe deficiency treatment. The pH of the nutrient solution started to decrease on the 8th day. On day 10, cultivars *ILC* 385 and *ILC* 8522 released maximum H^+ ions from the roots in response to Fe deficiency. The pH of the nutrient solution decreased from 6.5 to 2.9 and 3.9, respectively, while *ILC* 195 and *ILC* 8530 showed less response. This confirmed the findings of Moog and Bruggemann (1994) and Wei et al. (1995), who found that efficient cultivars exhibited an enhanced H^+ ion pump and were able to acidify the root medium by

increasing ATPase activity under Fe-deficiency stress conditions. Ellsworth et al. (1997) suggested that H^+ release and Fe reduction play a major role in the Fe efficiency of different cultivars of bean plants. A significantly higher activity of both H^+ release and Fe reduction was found in the roots of Fe-efficient cultivars. The decrease in the pH of the nutrient solution could be the result of the differential uptake of NH_4^+ and an increase in the ATPase activity in the plasmalemma of root cells (Landsberg, 1982; 1984; Kosegarten et al., 1999).

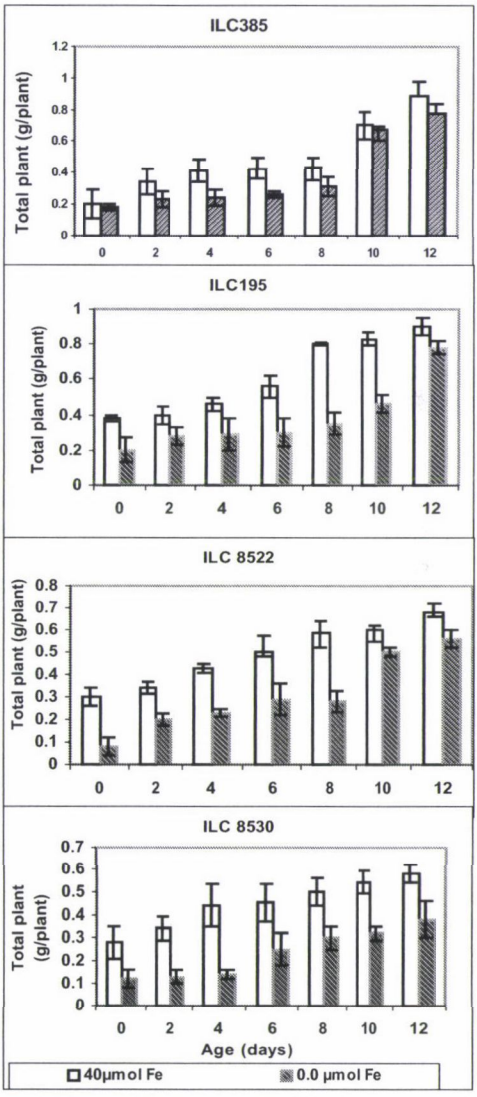

Fig. 1. Changes in total plant dry weight (g/plant) of ILC 385, 195, 8522 and 8530 chickpea cultivars grown in the presence or absence of 40 µmol Fe for 12 days. Bars indicate SD± value: standard deviation of three replicates

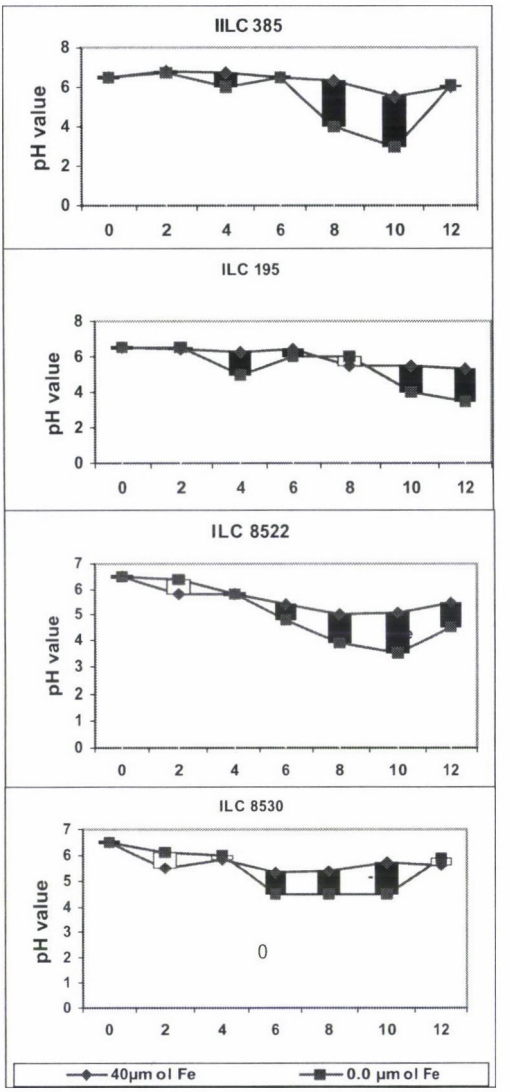

Fig. 2. Changes caused in the pH of the nutrient solution by the roots of ILC 385, 195, 8522 and 8530 chickpea cultivars grown in the presence or absence of 40 µmol Fe for 12 days

Fe reduction

It is interesting to note that chickpea cultivars differed in their ability to reduce $K_3[Fe(CN)_6]$ under Fe-deficient conditions. Cultivars *ILC* 385 and *ILC* 8522 showed the highest values (1.10 and 0.90 µM Fe^{2+}/g^{-1} F.W. h^{-1}), while cultivar *ILC* 195 had a moderate value (0.660 µM Fe^{2+}/g^{-1} F.W. h^{-1}) and cultivar *ILC* 8530 had the lowest Fe reduction capacity (0.555 µM Fe^{2+}/g^{-1} F.W. h^{-1}) in the treatment given no Fe (Fig. 3).

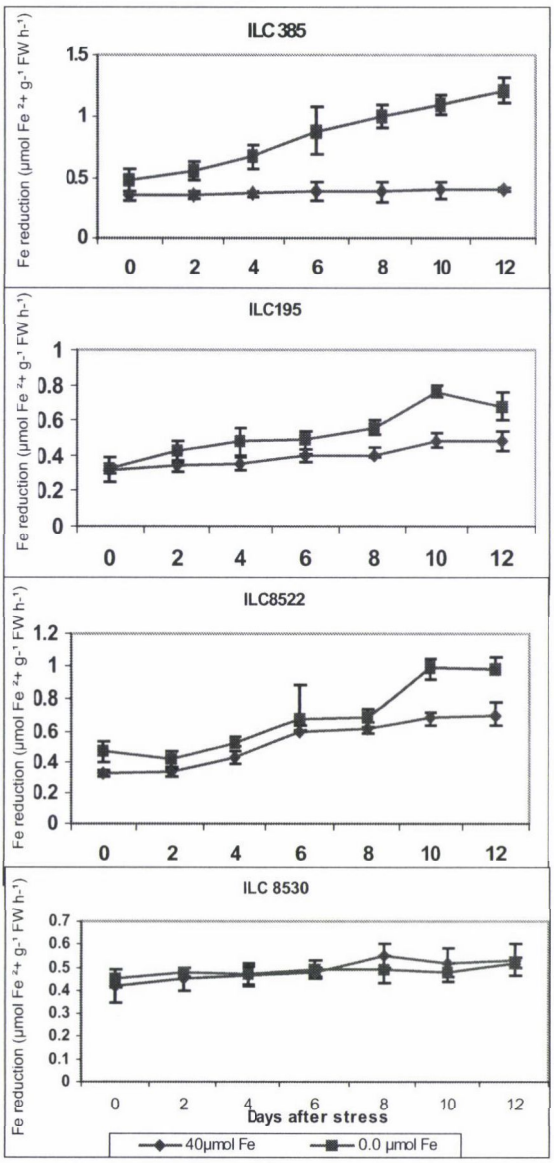

Fig. 3. Changes caused in Fe reduction by the roots of ILC 385, 195, 8522 and 8530 chickpea cultivars grown in the presence or absence of 40 µmol Fe for 12 days. Bars indicate SD± values: standard deviation of three replicates

It is well known that the reduction of Fe^{3+} to Fe^{2+} is necessary for the uptake of iron by plants (Camp et al., 1987; Kosegarten et al., 1999). It was concluded that the inheritance of resistance to Fe deficiency is controlled by a single recessive gene (Brian et al., 2002).

In conclusion, the results of the present study indicated that H^+ release and Fe reduction mechanisms can be considered as potentially reliable techniques for

predicting the ability of different plant species to survive iron deficiency. Further impetus could also be given to genetic improvement programmes for the selection of tolerant (Fe-efficient) genotypes.

Acknowledgements

This work was conducted as part of the Egyptian-German project "Micronutrient and Plant Nutrition Problems in Egypt", implemented by the National Research Centre, Cairo (Coordinator: Prof. M. M. El-Fouly) and the Institute of Plant Nutrition (Prof. A. Amberger). The project was supported by the Egyptian Academy of Scientific Research and Technology (ASRT) and the German Federal Ministry for Economic Cooperation and Development (BMZE) through the German Agency for Technical Cooperation (GTZ).

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Acta Agronomica Hungarica, 53(2), pp. 229–239 (2005)

GRADUAL INCREASE IN NaCl CONCENTRATION OVERCOMES INHIBITION OF SEED GERMINATION DUE TO SALINITY STRESS IN *SORGHUM BICOLOR* (L.)

F. A. FAHEED*, A. M. HASSANEIN* and M. M. AZOOZ**

* BOTANY DEPARTMENT, FACULTY OF SCIENCE, SOUTH VALLEY UNIVERSITY, SOHAG, EGYPT
** BOTANY DEPARTMENT, FACULTY OF SCIENCE, SOUTH VALLEY UNIVERSITY, QENA, EGYPT

Received: 22 March, 2004; accepted: 12 January, 2005

A gradual increase in NaCl concentration in the growth medium was used as a strategy to adapt sorghum plants (*Sorghum bicolor* L.) to relatively high concentrations of NaCl. Over a period of 15 days, a low percentage (22.2%) of sorghum seeds germinated in 200 mM NaCl, but most of the seedlings obtained (85.8%) died. On the other hand, plants subjected to adaptation by a gradual increase in NaCl concentration in the growth medium became capable of growth in soil containing 300 mM NaCl. In general, salinization induced a highly significant decrease in fresh and dry masses, and in the pigment content of sorghum seedlings. The content of free amino acids and soluble carbohydrates increased with a rise in the salinization level, especially in the adapted sorghum plants. The adapted plants contained less Na^+ but more K^+ compared to the unadapted plants, especially when the plants were subjected to relatively high NaCl concentration. Plants adapted in soil showed a new peroxidase isoenzyme form (POX-4). The peroxidase band POX-1 was detected under salt stress in both adapted and unadapted plants. Under salt stress, indophenol oxidase and glutamate oxaloacetate transaminase expressed new isoenzyme forms, IPOX-3 and IPOX-5, and GOT-2 and GOT-3, respectively. The induction of salt tolerance by a gradual increase in NaCl concentration for three weeks was recommended to overcome the inhibition of seed germination in saline soil.

Key words: adaptation, chemical analysis, isoenzymes, salinity stress, sorghum

Introduction

Salinity significantly inhibits seed germination and seedling growth due to the combined effects of low osmotic potential and ion toxicity (Grieve and Suarez, 1997; Katembe et al., 1998; Khan and Ungar, 1998). Soluble components of carbohydrates and protein might be of general importance in osmotic adjustment, and have been found to accumulate in some plants under salinity stress (Weimberg et al., 1984; Vallauri et al., 1989).

The ability of plants to grow under salinity stress increased when the plants were subjected to a gradual increase in NaCl concentration in the growth medium (Nasser et al., 1980; Wated et al., 1985). This type of adaptation takes longer to develop than osmotic adjustment (Amzallag et al., 1990), requiring about 50 generations in *Nicotiana*, which is attributed to the modulation in gene expression (Wated et al., 1985; Bizel et al., 1985).

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Reactive oxygen species are generated in all living cells during their normal metabolism. Under stress conditions, the overproduction of reactive oxygen species results in significant damage to cellular constituents or even cell death if detoxifying enzymes are not sufficient to convert them to harmless metabolites (Bowler et al., 1994; Polle, 1995; Paolacci et al., 1997; Hassanein, 1997; Hassanein et al., 1999; El-Tayeb and Hassanein, 2000). Consequently, studying peroxidase, indophenol oxidase and other antioxidative enzymes is an important step towards understanding the effect of abiotic stress on green plants. Since the synthesis of proline from carbohydrate via α-ketoglutrate and glutamate is the main source of proline accumulation under stress conditions (Boggess et al., 1976; Venekamp and Koot, 1988), transaminase enzymes (glutamate oxaloacetate transaminase) should be studied.

The aim of this work was to study how the oxidase-reductase and transaminase enzymes were affected by the application of a gradual increase in NaCl concentration in soil and water cultures in comparison with those in plants grown under the corresponding, relatively high NaCl concentrations. Since adaptation to salinity results from a sequence of physiological events, the growth, pigments, proline, soluble proteins and carbohydrates were also studied. A further aim was to discover whether plants treated with a gradual increase in NaCl concentration became adapted to growth in soil containing a relatively high concentration of NaCl, thus allowing this method to be used to overcome the inhibition of seed germination under stress conditions.

Materials and methods

Effect of osmotic stress on the seed germination and seedling growth of sorghum plants

Fifteen seeds of sorghum plants (*Sorghum bicolor* L.) were sown in plastic pots containing 600 g of air-dried clay soil supplemented with 0, 50, 100, 150, 200, 250 and 300 mM NaCl. The moisture of the soil was adjusted to full field capacity (26%). Salinized and non-salinized plants (three replicates) were irrigated every other day with distilled water to full field capacity. After two weeks, the percentage of seed germination was determined. Seeds were considered to be germinated after the emergence of the coleoptiles from the soil surface. After the seedlings were four weeks old, they were harvested, and their fresh and dry weights were determined. All the experiments were carried out under greenhouse conditions.

Adaptation in water culture

Seeds of sorghum were germinated in wetted sawdust using half strength Hoagland's solution (Hewitt, 1963). One-week-old seedlings were transferred to aerated half-strength Hoagland's solution containing 25 mM NaCl. The plants were subjected to a gradual increase in NaCl concentration by transferring them every 2 days to new solution containing the next higher concentration (50, 75, 100, 125, 150, 200, 225, 250, 275 and 300 mM NaCl). Samples from each concentration (three replicates) were rapidly washed in tap water and dried to determine fresh and dry weights as well as to carry out certain analyses.

Adaptation in soil

Deep trays containing soil (10 cm depth, 26% field capacity) were used in this experiment. One hundred seeds were sown in each. Salinization commenced on the 8th day by supplementing each tray with 25 mM NaCl, after which the concentration of NaCl was increased by 25 mM NaCl every two days. At high concentrations of NaCl, the dead plants were removed. Plants subjected to 50, 100, 150, 200, 250 and 300 mM NaCl (three replicates) were harvested to determine fresh and dry masses as well as to carry out the chemical analyses.

Chemical analysis

The photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) of the plants were determined using the spectrophotometric method recommended by Metzner et al. (1965). The dry matter yields were used (three replicates) for chemical analysis. For the determination of water-soluble sugars, a known weight of the powdered tissue was hydrolysed in distilled water for two hours in a boiling water bath. After cooling, the hydrolysate was filtered and the filtrate was made up to a known volume, after which the water-soluble saccharides were determined by the anthrone sulphuric acid method (Fales, 1951).

Free amino acids were extracted from the plant tissues and determined according to the method of Moore and Stein (1948), while free proline was determined according to Bates et al. (1973).

To estimate the soluble proteins, powdered tissue samples were boiled in distilled water for two hours. After cooling, the water extract was centrifuged and the supernatant was decanted and made up to a known volume with distilled water. The insoluble protein residue remaining after the extraction of the water-soluble fraction was treated with NaOH. The plant protein content was determined according to Lowry et al. (1951).

Sodium and potassium were determined by the flame photometer method (Williams and Twine, 1960) using an instrument of the Carl Zeiss type.

Isoenzyme analysis

One gram of plant leaves subjected to a gradual increase in NaCl concentration up to 200 mM NaCl in soil or water for 20 days (25 mM every 3 days) or 10 days (25 mM each day for 4 successive concentrations, then 25 mM every 2 days) was ground at 4°C in a mortar in 1 cm^3 of extraction buffer consisting of 0.1 M Tris-HCl, pH 7.0, containing 0.002 M cysteine. The homogenate was centrifuged at 15,000 g at 4°C for 15 min. The supernatants were collected for immediate electrophoresis in 7.5% polyacrylamide slab gels. The samples contained 40 µg protein. The gels were run at 18 mA for 6 h at 10°C in 0.025 M Tris+0.192 M glycine buffer (pH 8.9). Four isoenzymes were stained: peroxidases (Siegel and Galston, 1967), esterases (Wetter and Dyck, 1983; Brewer, 1970), indophenol oxidase and glutamate oxaloacetate transaminase (Brewer, 1970).

Results and discussion

The results obtained for the germination of sorghum seeds subjected to various NaCl treatments are given in Table 1. In comparison to plants grown under non-stress conditions (control), 50 mM NaCl resulted in a significant increase in germination percentage, but a further increase in the NaCl concentration to above 50 mM resulted in a significant reduction in seed germination. In soil containing 200 mM NaCl a large number of seedlings died after emergence from the soil, and even those which started to develop often died later; consequently, the number of plants at harvest, after 4 weeks, was lower (8%) than the number of germinated seeds (22.2%). The inhibitory effect of NaCl on seed germination and seedling growth may be attributed to the combined effect of low osmotic potential and specific ion toxicity (Bernstein, 1993; Grieve and Suarez, 1997; Katembe et al., 1998, Khan and Ungar, 1998). The present work indicates that seedling growth was more sensitive to salt stress than seed germination, because many germinated seeds were unable to tolerate the osmotic stress exerted by 200 mM NaCl and died within a few days. This may have been due to the accumulation of Na^+ and Cl^- in the seedling tissues, finally leading to death (Amzallag et al., 1990).

Table 1
Effect of different NaCl concentrations (mM) on seed germination (%) of *Sorghum bicolor* plants

NaCl	Germination
0 (Control)	86.67
50	97.77*
100	68.90**
150	35.43**
200	22.20**
250	00.00
$L.S.D._{5\%}$	7.83
$L.S.D._{1\%}$	11.13

*Significant differences ; ** Highly significant differences

The fresh and dry weights of sorghum plants (Table 2) decreased only slightly at 50 mM NaCl, but thereafter the values of these parameters decreased significantly compared with the control. The growth of 22-day-old seedlings subjected to 200 mM NaCl after a gradual increase in the NaCl concentration in water or in soil improved in comparison to that of seedlings germinated in soil containing the same concentration of NaCl. Furthermore, sorghum plants could tolerate a soil salt content of 300 mM NaCl and continue to grow when this concentration was reached step by step, at the rate of 25 mM every two days. These data indicated that a gradual increase in the NaCl concentration in the growth medium increased the salt tolerance of sorghum seedlings. Similar results were obtained by Amzallag et al. (1990), who considered this behaviour as the adaptation of sorghum to salinity stress. The data also indicated that a gradual increase in the NaCl concentration was more effective in soil than in water culture, since seedlings were only able to grow in the presence of 300 mM NaCl in soil.

Seedlings adapted to salt stress by a gradual increase in the NaCl concentration up to 300 mM for 22 days were transplanted to other pots containing 300 mM NaCl in a culture room. The adapted plants remained alive for 4 days. On the other hand, non-adapted seedlings wilted in a few hours (4 h) when they were suddenly exposed to a relatively high concentration of NaCl (300 mM). This indicated that subjecting the plants to a gradual increase in NaCl concentration for three weeks allowed them to tolerate relatively high concentrations of NaCl. The adapted plants may need acclimation under plastic bags or under mist to conserve high humidity during the first three days, like plants obtained from tissue culture, if they are to be able to continue growth after transplantation, or it may be necessary to conserve the complete root system during eradication (this will be the subject of a separate study on sorghum and other plants).

The gradual increase in the NaCl concentration was commenced when the seedlings were 8 days old and reached 300 mM NaCl at 30 days. The transplanting of the adapted plants to new pots containing a high NaCl concentration was aimed at avoiding the inhibition of seed germination under relatively high salt stress (250 and 300 mM NaCl). An increase in the salt tolerance with age was reported in many plants including sorghum (Maas, 1986; Maas et al., 1986). Amzallag et al. (1990) reported that the adaptation of sorghum seedlings due to NaCl pretreatment cannot be ascribed to the age of the plants.

There was a slight rise in the contents of chl. a, chl. b, carotenoids and consequently total pigments (Table 3) when increasing the level of NaCl to 100 mM, but a pronounced decrease was observed in growth and pigments at higher salinity levels, as in other reports (Hernandez et al., 1995; Fricke and Perters, 2002). The pigment contents of plants subjected to salt stress via a gradual increase in NaCl concentration (data not shown) were the same as in plants grown under the corresponding NaCl concentrations without adaptation.

Table 2

Effect of different NaCl concentrations (mM) on fresh and dry matter (g plant^{-1}) of *Sorghum bicolor* grown in saline soil or subjected to the same concentrations after a gradual increase (25 mM/2 days) in NaCl concentration

Treatments	Seedling age (day)	NaCl	Fresh weight	Dry weight
Non-adapted plants	22	0 (Control)	0.28	0.027
	22	50	0.25	0.025
	22	100	0.20**	0.023*
	22	150	0.12**	0.018**
	22	200	0.08**	0.016**
	$L.S.D._{5\%}$		0.04	0.003
	$L.S.D._{1\%}$		0.06	0.005
Adaptation in water	8	Control	0.138	0.014
	10	50	0.17*	0.022**
	14	100	0.197**	0.025**
	18	150	0.13	0.027**
	22	200	0.178**	0.032**
	$L.S.D._{5\%}$		0.03	0.007
	$L.S.D._{1\%}$		0.05	0.008
Adaptation in soil	8	Control	0.11	0.01
	10	50	0.16*	0.015**
	14	100	0.20**	0.020**
	18	150	0.158*	0.018**
	22	200	0.178**	0.019**
	26	250	0.177**	0.018**
	30	300	0.17**	0.025**
	$L.S.D._{5\%}$		0.05	0.002
	$L.S.D._{1\%}$		0.06	0.003

*Significant differences; ** Highly significant differences

Table 3
Effect of different NaCl concentrations (mM) on pigment contents (mg g^{-1} fresh weight) of *Sorghum bicolor* plants

NaCl	Chl. A	Chl. B	Carot.	Total
0(Control)	1.980	0.835	0.614	3.429
50	1.891	0.748*	0.544	3.183
100	2.046	0.856	0.639	3.543
150	1.767	0.664**	0.533	2.967**
200	1.440**	0.506**	0.534	2.481**
$L.S.D._{5\%}$	0.36	0.08	0.136	0.288
$L.S.D._{1\%}$	0.50	0.11	0.201	0.404

*Significant differences; ** Highly significant differences

Bernstein (1993) reported that green plants could adjust osmotically to about 24 mM NaCl per day by increasing the concentration of internal solutes. The present data support this conclusion, since the soluble carbohydrate contents increased at all the applied concentrations of NaCl (Table 4). The highest increase in soluble carbohydrates was detected when the plants were subjected to relatively high concentrations of NaCl (100–200 mM). Higher contents of soluble carbohydrates were generally exhibited by plants subjected to adaptation, especially in soil, in comparison with unadapted plants. This indicates that adaptation may improve photosynthesis.

To survive under salinity stress, plants may protect themselves by producing high internal concentrations of organic solutes to lower the osmotic potential of the cell or accumulate ions in response to high concentrations of salt in their environment (Faheed, 1993; Piqueras et al., 1996; Bavenport et al., 2003; Abraham et al., 2003). Under salt stress sorghum plants accumulated soluble carbohydrates (Table 4), since all the NaCl treatments resulted in an increase in the soluble carbohydrate and free amino acid contents. The soluble protein content of adapted seedlings was slightly higher in comparison to that of non-adapted seedlings. On the other hand, free amino acids and total protein were both higher in plants subjected to a gradual increase in NaCl concentration up to 200 mM, especially in soil. The increase in nitrogen-containing compounds may play an important role in adjusting the osmotic potential, in protecting cellular macromolecules, in maintaining cellular pH, and in scavenging free radicals (Mansour, 2000).

A significant accumulation of proline content was recorded after the sorghum plants were subjected to all the NaCl treatments used. The proline content of seedlings adapted to growth under salt stress was the same as that of plants subjected to the corresponding NaCl concentrations without adaptation (Table 5).

Table 4
Effect of different NaCl (mM) concentrations on the content of soluble carbohydrates, soluble proteins and total free amino acids (mg g^{-1} dry matter) of *Sorghum bicolor* grown in saline soil or subjected to the same concentrations after a gradual increase (25 mM/2 days) in NaCl concentration

Treatments	Seedling age (day)	NaCl	Soluble carbohydrates	Soluble proteins	Free amino acids	Total protein
Non-adapted plants	22	0 (Control)	12.25	41.47	1.86	53.70
	22	50	19.22*	50.05**	2.12	78.01**
	22	100	30.90**	45.25	2.22	70.29**
	22	150	54.32**	46.68*	2.55	53.26
	22	200	53.49**	46.20*	2.75	61.34
Adaptation in water	10	50	66.94**	73.19**	2.08	94.25**
	14	100	50.29**	56.54**	3.31*	84.33**
	18	150	56.42**	44.66	3.20*	65.84*
	22	200	48.34**	53.86**	3.17*	67.12**
Adaptation in soil	10	50	90.72**	48.80**	8.32**	76.73**
	14	100	73.79**	45.39	4.16**	100.47**
	18	150	88.61**	46.88*	3.59*	105.93**
	22	200	100.38**	46.12*	3.29*	105.19**
	26	250	71.04**	35.27**	2.85	56.40
	30	300	75.02**	24.95**	2.05	40.26**
$L.S.D._{5\%}$			7.58	4.67	1.26	9.56
$L.S.D._{1\%}$			10.67	6.57	1.78	13.29

*Significant differences; ** Highly significant differences

Table 5
Effect of different NaCl concentrations (mM) on proline content (mg g^{-1} dry weight) of *Sorghum bicolor* plants

NaCl	Proline
0 (Control)	0.32
50	0.71**
100	0.59*
150	0.71**
200	1.16**
$L.S.D_{5\%}$	0.26
$L.S.D_{1\%}$	0.37

*Significant differences; ** Highly significant differences

The sodium content of plants subjected to salt stress was higher than that of the control plants (Table 6). On the other hand, the K^+ content decreased with an increase in the NaCl concentration. The adaptation of plants due to a gradual increase in NaCl concentration resulted in a decrease in Na^+ but an increase in K^+ contents in comparison to those of plants subjected to the corresponding NaCl concentrations without adaptation. This may thus be the mechanism used by the plant to partially overcome the toxic effect of NaCl during salt stress. Adaptation to salinity stress is more than just osmotic adjustment and takes longer to develop. It comprises the development of a capacity to regulate internal Na^+ and Cl^- concentrations, even when external salinity is high (Amzallag et al., 1990; Lee et al., 2004).

Table 6
Effect of different NaCl (mM) concentrations on the Na^+ and K^+ contents (mg g^{-1} dry weight) and Na^+/K^+ ratio of *S. bicolor* grown in saline soil or subjected to the same concentration after a gradual increase (25 mM/2 days) in the NaCl concentration

Treatments	Seedling age (day)	NaCl	Na^+	K^+	K^+/Na^+
Non-adapted plants	22	0 (Control)	0.19	8.62	45.36
	22	50	0.28**	7.35**	26.25**
	22	100	0.32**	7.23**	22.59**
	22	150	0.35**	6.99**	19.41**
	22	200	0.37**	6.19**	17.19**
Adaptation in water	10	50	0.26**	12.39*/*	47.65
	14	100	0.27**	11.54**	42.74
	18	150	0.28**	9.73**	34.75*
	22	200	0.33**	11.11**	33.66*
Adaptation in soil	10	50	0.27**	12.22**	101.80**
	14	100	0.27**	10.91**	40.40
	18	150	0.29**	10.71**	36.93
	22	200	0.32**	10.33**	32.81*
	26	250	0.33**	10.09**	31.53**
	30	300	0.33**	10.09**	31.53**
$L.S.D._{5\%}$			0.02	0.49	9.3
$L.S.D._{1\%}$			0.05	0.68	13.6

*Significant differences; ** Highly significant differences

The expression patterns of isoenzymes displaying peroxidase activity (Fig. 1A) were very similar in plants obtained from seeds germinated in soil containing 200 mM NaCl and in those subjected to a gradual increase in NaCl concentration up to 200 mM where they expressed a new isoenzyme form (POX-1). A low staining intensity of POX-1 was detected when the seedlings were adapted for a short period (10 days). Plants subjected to adaptation for 20 days in soil showed the expression of POX-4, but this band was not detected in plants subjected to other treatments. This indicates that NaCl regulates the expression of this isoenzyme form.

The isoenzyme profile of indophenol oxidase (Fig. 1B) showed that salinity stress induced the expression of two new isoenzyme bands (IPOX-3 and IPOX-5) and increased the staining intensity of a third one (IPOX-2). On the other hand, a gradual increase in the NaCl concentration led to the disappearance of one band (IPOX-6).

The malate dehydrogenase pattern (Fig. 1C) was not influenced by salt stress, but the staining intensity of the MDH-1, MDH-3 and MDH-4 bands decreased in plants subjected to a gradual increase in the NaCl concentration in soil.

The studied isoenzymes, peroxidase, indophenol oxidase and malate dehydrogenase, may prevent the accumulation of H_2O_2 through NADH-POX activity (De Marco and Roubelakis-Angelakis, 1996a, b; Hassanein et al., 1999). During protoplast regeneration, a parallel enhancement in cell wall-bound

malate dehydrogenase and NADH-POX activities were reported (De Marco and Roubelakis-Angelakis, 1996a; Simins et al., 1993). Malate dehydrogenase plays a role in scavenging the reactive oxygen species generated under salt stress (Bavenport et al., 2003), via the activation of the malate valve (Polle, 1995).

The number of GOT bands (Fig. 1D) increased when the plants were subjected to salt stress, especially in the case of 200 mM NaCl for 20 days. The two extra isoenzyme bands (GOT-2 and GOT-3) were also expressed in plants subjected to 200 mM NaCl for 10 days, but with low staining intensity. GOT enzymes may play an important role in increasing the content of proline under salt stress (Boggerss et al., 1976; Venekamp and Koot, 1988).

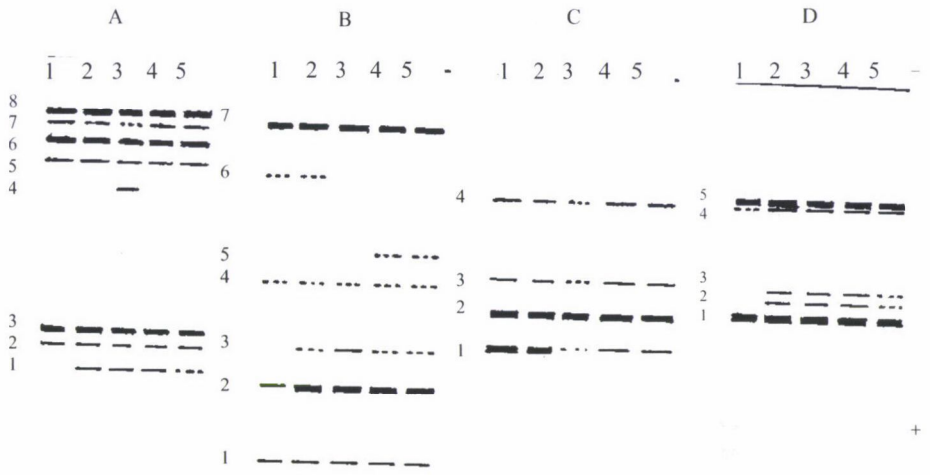

Fig. 1. Native gel electrophoresis of peroxidase (A), indophenol oxidase (B), malate dehydrogenase (C) and glutamate oxaloacetate transaminase (D) in sorghum plants grown under salt stress in comparison to those of control plants (Lane 1). Lane 2: plants grown for three weeks in soil containing 200 mM NaCl. Lanes 3, 4 and 5: plants subjected to a gradual increase in NaCl concentration up to 200 mM Nacl in soil within three weeks (Lane 3) and in water culture within three weeks (Lane 4) or 10 days (Lane 5). (━ dark stained band, - intermediate stained band, ┄ light stained band

Conclusions

A gradual increase in the NaCl concentration in the soil (25 mM every 2 days) for three weeks or more helped sorghum plants to adapt to growth under relatively high salt stress. Consequently, these plants could be grown in soil containing high NaCl concentrations which completely prevented seed germination. More investigations will be required to evaluate the suitability of adaptation via a gradual increase in NaCl concentration for cultivation on saline soils. Plants tolerant of transplantation would be recommended.

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ISSN 0238-0161

Printed in Hungary
PXP, Budapest

Acta Agronomica Hungarica

An International Multidisciplinary Journal in Agricultural Science

VOLUME 53, NUMBER 3, 2005

Akadémiai Kiadó, Budapest

MEMBER OF WOLTERS KLUWER GROUP

ACTA AGRONOMICA HUNG. AAHUEX 53 (3) 241–358 (2005) HU ISSN 0238-0161

ACTA AGRONOMICA HUNGARICA

A QUARTERLY OF THE HUNGARIAN ACADEMY OF SCIENCES

Acta Agronomica Hungarica publishes papers in English on agronomical subjects, mostly on basic research

Acta Agronomica Hungarica is published in yearly volumes of four issues by

AKADÉMIAI KIADÓ, Budapest, Hungary

Acta Agronomica Hungarica is abstracted/indexed in Biological Abstracts, BIOSIS Previews, CAB Abstracts, Chemical Abstracts, GEOBASE, Global Health, Index Veterinarius, International Bibliographics IBZ and IBR

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The Agricultural Research Institute of the Hungarian Academy of Sciences contributes financially to the publication of *Acta Agronomica Hungarica*.

AAgr 53 (2005) 3

ACTA AGRONOMICA HUNGARICA

AN INTERNATIONAL MULTIDISCIPLINARY JOURNAL IN AGRICULTURAL SCIENCE

VOLUME 53, NUMBER 3, 2005

AKADÉMIAI KIADÓ, BUDAPEST

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ORIGINAL PAPERS

Acta Agronomica Hungarica, 53(3), pp. 241–250 (2005)

EFFECTS OF A CARBONIZATION PRODUCT AS ADDITIVE ON THE GERMINATION, GROWTH AND YIELD PARAMETERS OF AGRICULTURAL CROPS

K. SVETKOVÁ[1], M. HENSELOVÁ[2] and M. MORVOVÁ[3]

[1]FACULTY OF CHEMICAL AND FOOD TECHNOLOGY, SLOVAK UNIVERSITY OF TECHNOLOGY, BRATISLAVA, SLOVAKIA
[2]FACULTY OF NATURAL SCIENCES, COMENIUS UNIVERSITY, BRATISLAVA, SLOVAKIA
[3]FACULTY OF MATHEMATICS, PHYSICS AND INFORMATICS, COMENIUS UNIVERSITY, BRATISLAVA, SLOVAKIA

Received: 19 April, 2005; accepted: 19 September, 2005

The effects of the carbonization product of separated communal waste as an additive to the soil was studied on the germination, quantitative growth and yield parameters of maize (*Zea mays* L.), bean (*Phaseolus vulgaris* L.) and tomato (*Solanum lycopersicum* L.). The product was tested on three soil types with different humus content and mineral composition in doses of 1, 10 and 20 g of additive per kg soil, with application before seeding or after plant emergence, under greenhouse and field conditions. It was found that plants utilized the nutrient content from the carbonization products in both methods of application and that the stimulatory effect on the germination, fresh and dry mass and yield parameters of the treated plants was dependent on the plant species, soil type, dose and method of application. A significant effect of the additive was found on the germination of bean, which increased by 8–22% over the control. Fresh and dry mass increased by 18–62% in maize and by 2–30% in bean when the additive was applied under greenhouse conditions. In the field the additive was found to have a positive effect on the average mass of tomato fruits and maize cobs, and on the yield per plant.

Key words: non-traditional fertilizer, carbonization product, maize, bean, tomato, germination, fresh and dry mass, yield

Introduction

One of the main factors in the intensification of plant production is the appropriate fertilization of plants, which decides the quantity and quality of many agricultural crops (Ložek, 2001; Ankumah et al., 2003; Hlušek et al., 2003; Liua et al., 2003; Márton, 2004). Although the current spectrum of industrial fertilizers in the Slovak Republic is already extensive (Vaneková, 1989; Fecenko and Ložek, 2000), the development of new fertilizers continues (Jahnátek, 2003). This generally depends on the agricultural production and economic potential of individual countries. Nowadays many new and non-traditional fertilizers are being tested. Proteinaceous hydrolysate prepared from tanning waste stimulates the germination and growth of maize, barley and cucumber (Bezák et al., 1989). Zeolite fertilizer (Zeomix) increases the yield of spring barley (Rimár, 1999). Anaerobically digested grey municipal solid waste can serve as a readily available N source to ensure rapid crop development (Makaly Biey et al., 2000). A mixture of organomineral complexes with morphoregulatory and antistress effects shows positive effects on the increase of dry mass in winter wheat (Hudec et al., 2001).

0238–0161/$ 20.00

One alternative source potentially utilizable in plant nutrition is the product of the carbonization of separated solid communal waste. Waste has become a critical problem for industrialized society, particularly in big cities and densely populated areas (Grodziska-Jurczak, 2001; Fehr et al., 2002). Therefore new solutions for waste treatment need to be elaborated. It requires research and development throughout the world. According to Bedő (2003), in agriculture, too, only the comprehensive handling of ecological, economic and social challenges can produce a satisfactory answer to the questions involved in sustainable development.

The present study summarizes the results of greenhouse and small-plot field investigations on the carbonization product of communal waste as an additive to the soil. The effects of the additive were examined on the germination and some quantitative growth and yield parameters of three agricultural crops, maize (*Zea mays* L.), bean (*Phaseolus vulgaris* L.) and tomato (*Solanum lycopersicum* L.).

Materials and methods

Preparation of additive

The additive was prepared by carbonizing separated solid communal waste (plant and animal residues) at the Faculty of Mathematics, Physics and Informatics of Comenius University, Bratislava. Carbonization is a thermo-chemical degradation process in the absence of oxygen, so that carbon char is formed instead of ash. A detailed description of the process, additive preparation and its physical properties are described by Morvová et al. (2003). The results of the chemical analysis of the additive are presented in Table 1.

Plant material and soil

Biological research on the additive was done on maize (*Zea mays* L. cv. Torena), bean (*Phaseolus vulgaris* L. cv. Unidor F_1) and tomato (*Solanum lycopersicum* L. cv. Tornado). The additive was tested in three soil types (Table 2), which were analysed at the Research Institute of Soil Science and Conservation, Bratislava, where the chemical analysis of the additive was also performed. Soil reaction $pH_{(KCl)}$ was determined according to ISO 10390, carbon (oxidisable) and humus according to ISO/FDIS 14235, hydrolysis of samples according to Kjeldahl, content of mineral elements (Na, K, Ca, Mg, Fe) according to methods F-AAS ISO/DIS 14 869:1998 and ISO/FDIS 11 047:1997, phosphorus by the colorimetric method and total nitrogen according to STN ISO 11261.

Table 1
Chemical analysis of the additive, carried out at the Research Institute of Soil Science and Conservation in Bratislava, Slovak Republic

pH_{KCl}	C_{ox} (%)*	Humus (%)	Content of elements in additive ± SE (g kg^{-1})						
			$N_{Tot.}$	P	Na	K	Ca	Mg	Fe
8.7±0.3	6.3±0.6	10.8±1.0	15.9±1.5	7.1± 0.7	13.4±1.3	14.8±1.4	29.0±2.9	37.7±3.7	25.3±2.5

* Oxidisable carbon

Table 2
Agrochemical analysis of soil types, carried out at the Research Institute of Soil Science and Conservation in Bratislava, Slovak Republic

Soil type	pH	Concentration of elements in soil (mg kg^{-1})*				Humus (%)
		P	K	Mg	Ca	
I	6.6±0.2	39	150	575	11150	8.6±0.9
II	7.8±0.3	12	95	240	15400	0.9±0.1
III	7.7±0.3	644	2285	840	6100	3.8±0.4

* The standard error of the values did not exceed 10%

Characteristics of tested soils

Soil type I had a neutral soil reaction, a low content of available phosphorus, a medium content of available potassium and a very high content of available magnesium and calcium. The content of humus was very high. Soil type II had a highly alkaline soil reaction, a very low content of available phosphorus, a low content of available potassium, a high content of available magnesium and a very high content of available calcium. The content of humus was very low. Soil type III had an alkaline soil reaction with a very high content of all available nutrients (phosphorus, potassium, magnesium and calcium) and a relatively high content of humus.

Greenhouse experiments

The greenhouse experiments were carried out at the Department of Plant Physiology, Faculty of Natural Sciences of Comenius University, Bratislava. The effect of applying the additive before seeding (pre-emergence application) and after planting (post-emergence application) was tested on maize and bean. Twenty seeds of each species were sown in experimental pots containing soil types I and II mixed with the additive in doses of 1 and 20 g kg^{-1}, while the control was soil without additive. In the post-emergence application, pre-grown maize plants at the 2–3-leaf stage and bean at the 1st true primary leaf stage were individually planted in pots with the required amount of additive. Each treatment was carried out in three replications. Both treated and untreated (control) plants were irrigated daily. The influence of the additive on germination was evaluated ten days after sowing, and on the fresh and dry mass of the plants after seven weeks. Fresh and dry mass was evaluated according to Erdelský and Frič (1979).

Field experiments

The field trials were carried out in type III soil at Tvrdošovce (Nové Zámky district, South Slovakia). Before sowing maize or planting tomato, the soil was treated by traditional mechanical means. One kg of soil containing the requisite doses of 1, 10 and 20 g additive per kg soil was distributed in 1 m long rows before sowing maize. In the tomato experiments, 1 kg of soil with the required doses of additive was distributed under the root system of the tomato seedlings at a spacing of 450 × 500 mm. Every variant, with five plants per species, was replicated five times. During the growing season the plants were regularly irrigated. The average number and mass of tomato fruits and maize cobs and the total yield per plant were evaluated.

Evaluation of results

The effect of the additive on germination and on quantitative growth and yield parameters was evaluated. The statistical treatment of the data was done by calculating means ± SE and using Student's t-test.

Results

When the additive was applied to soil types I and II before sowing no significant effect was observed on maize germination under greenhouse conditions. Germination ranged from 99 to 106% of the control in soil type I and from 94 to 100% in soil type II. By contrast, the effect of the additive on bean germination was positive, giving a significant increase of 8–22% over the control.

The mass of maize plants was not positively influenced by applying the additive before sowing (Fig. 1A) and ranged from 92 to 95% of the control for fresh mass (i.e. 6.0–6.2 g per plant) and from 84 to 99% for dry mass (i.e. 0.66–0.78 g per plant) in soil type I, and from 96 to 97% (i.e. 4.71–4.75 g per plant) for fresh mass and from 85 to 102% (i.e. 0.56–0.68 g per plant) for dry mass in soil type II. In both soil types a dose of 20 g kg^{-1} of the additive was found to have a retarding effect on the dry mass, which decreased to 15–16% below the control with an average dry mass of 0.66–0.79 g per plant.

In the post-emergence application of the additive to maize a significant stimulatory effect was recorded in both soil types for fresh and dry mass production (Fig. 1B). In soil type I, which had higher humus content (8.2%) and essential elements, the stimulatory effect of the additive was observed at a dose of 20 g kg^{-1} of soil (Table 2). The fresh mass was significantly increased by 62%, with an average mass of 27.45 g per plant, and the dry mass by 56% with 3.39 g per plant. In the control the average fresh mass of maize was 16.92 g and the dry mass 2.16 g per plant. However, in soil type II, which had a very low content of humus (0.7%) and elements, the stimulatory effect of the additive was observed at the lower applied dose of 1 g kg^{-1}. The fresh and dry mass were significantly increased by 35 and 45% (15.94 g of fresh and 2.12 g of dry mass per plant) over the control, with an average fresh mass of 11.8 g and dry mass of 1.46 g per plant in the control. In soil type II at a dose of 20 g kg^{-1} of additive, the fresh and dry mass dropped to 18 and 23% below the control, respectively (Fig. 1B).

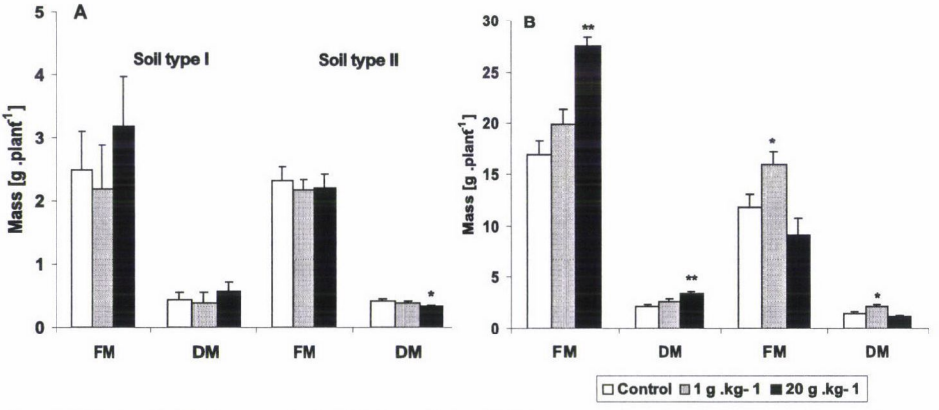

Fig. 1. Effect of additive on fresh (FM) and dry (DM) mass of maize (*Zea mays* L. cv. Torena) after pre-emergence (A) and post-emergence (B) application, determined in soil types I and II under greenhouse conditions. Data represent means ± SE (n=3).* Significant difference at P=0.05, ** Significant difference at P=0.01

The pre-emergence application of the additive to bean (Fig. 2A) in soil type I only had a positive effect on the fresh and dry mass at a dose of 20 g kg^{-1}, where the fresh mass increased by 28% (3.19 g) and the dry mass by 30% (0.56 g) compared with 2.49 g per plant fresh mass and 0.43 g dry mass in the control. In soil type II no positive effect on growth and quantitative parameters was observed. Fresh mass in beans ranged from 2.16 to 2.21 g per plant (93–95% of the control) and dry mass from 0.33 to 0.38 g per plant (79–92% of the control). At a dose of 20 g kg^{-1} in soil type II dry mass decreased significantly by about 21%, from 0.41 g per plant in the control to 0.33 g per plant (Fig. 2A). In post-emergence application the effect of the additive on bean growth was only positive at the higher dose of 20 g kg^{-1}. The fresh mass in soil types I and II increased significantly by 17 and 24% and dry mass increased insignificantly by 4 and 13% in comparison to the control, with fresh mass values of 3.61 to 5.31 g and dry mass values of 0.67 to 1.0 g per plant (Fig. 2B). The dry to fresh mass ratio in both species was enhanced under the greenhouse conditions, indicating water loss from treated and untreated maize and bean plants.

The results of field experiments carried out in soil type III (location: Tvrdošovce) showed that the total yield of maize cobs per plant was not influenced positively by the additive (Fig. 3A). It ranged from 102.9 to 118.6 g per plant, with 118.7 g per plant in the control. The number of cobs per plant decreased to 76–81% of the control (Fig. 3B), though the average cob mass increased significantly by 7–31% over the control.

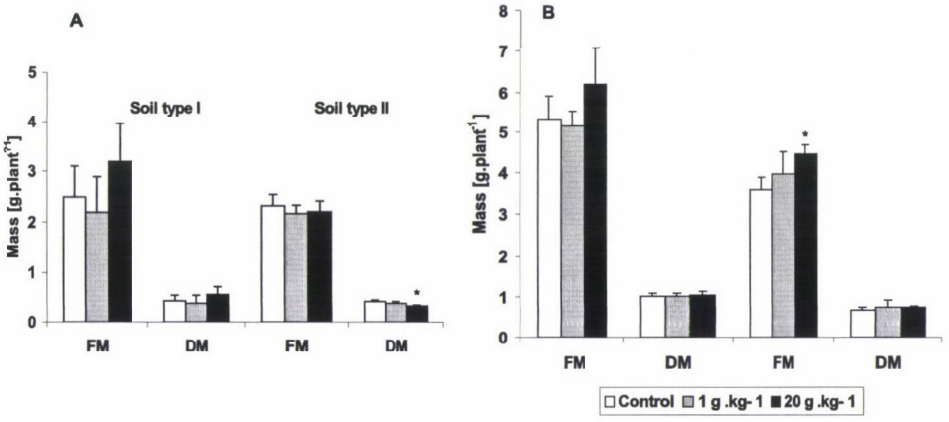

Fig. 2. Effect of additive on fresh (FM) and dry (DM) mass of bean (*Phaseolus vulgaris* L. cv. Unidor F_1) after pre-emergence (A) and post-emergence (B) application, determined in soil types I and II under greenhouse conditions. Data represent means ± SE (n=3). *Significant difference at P= 0.05

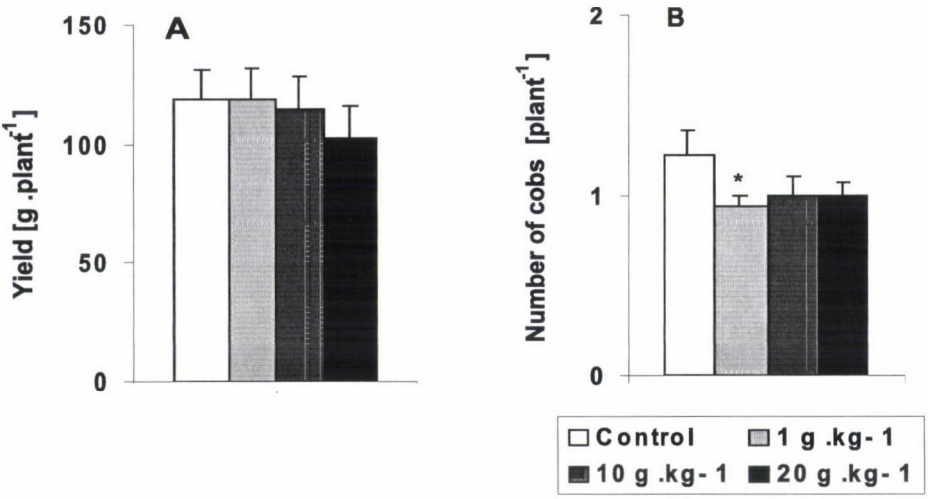

Fig. 3. Effect of additive on yield (A) and number of cobs (B) per plant of maize (*Zea mays* L. cv. Torena) determined in soil type III in Tvrdošovce. Plants were cultivated under field conditions from May 4 to October 6, 2001. Data represent means ± SE (n=5). *Significant difference at P= 0.05

In tomato plants the number of fruits per plant over the whole season (Fig. 4B) depended on the dose. At 1 g kg^{-1} the positive effect was about 12% over the control with 38.3 fruits per plant, but at 20 g kg^{-1} the additive had a negative effect, with 26.4 fruits per plant, which was 23% less than the control, where there were 34.2 fruits per plant. The influence of the additive on the total yield of tomato fruits per plant was similar (Fig. 4B). The additive had a positive effect on the number of fruits per plant and on the total yield in the first two harvests (Fig. 4A). The increase in the number of fruits per plant was 10% (6.8 fruits per plant) at 20 g kg^{-1} and 42% (8.8 fruits per plant) at 10 g kg^{-1}, while the total yield increased by 13% (253 g per plant) and 50% (337 g per plant), respectively, over the control, where 6.2 fruits and a yield of 224 g per plant were recorded after the first two harvests (Fig. 4A).

Discussion

At present, the production of good quality food products is impossible without intensification factors such as fertilizers that influence the yield potential of agricultural crops (Novoa and Loomis, 1981; Fecenko and Ložek, 2000; Kismányoky and Ragasits, 2003; Ramachandrappa et al., 2004). According to Bujnovský et al. (1998), the requirements of crops for essential elements are supplied from many sources, e.g. soil reserves, application of organic fertilizers, fixation of nitrogen by microorganisms. The tested additive, which is a product

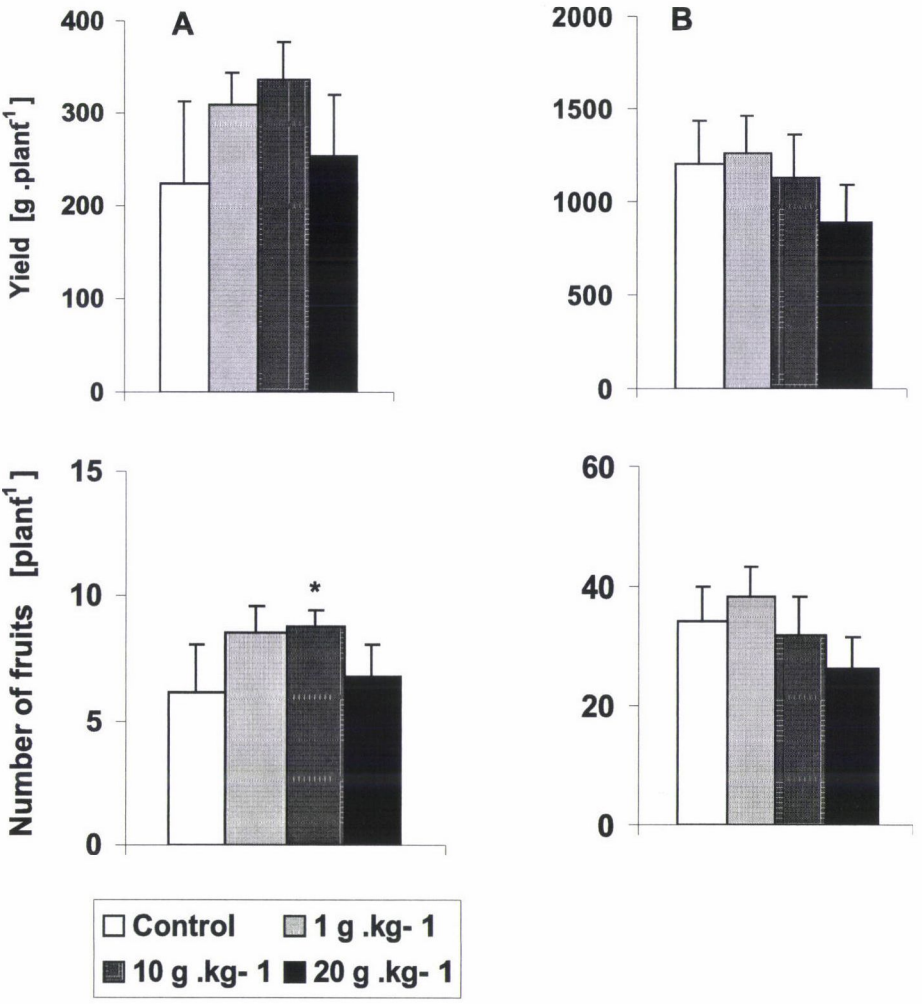

Fig. 4. Effect of additive on yield and number of fruits per plant of tomato (*Solanum lycopersicum* L. cv. Tornado) determined in soil type III in Tvrdošovce. Plants were cultivated under field conditions from May 5 to September 23, 2001. The first two harvests were made on July 8 and 15, 2001. Data represent means ± SE (n=5). A = Parameters after the first two harvests / per plant B = Parameters over the whole growing season / per plant * Significant difference at P= 0.05

of the carbonization of plant and animal food wastes, ranks among the non-traditional and as yet unverified sources of prospective plant fertilizer. The method developed for the carbonization of communal waste offers an effective way of utilizing it. Infrared spectrum analysis (Svetková, 2002; Morvová et al., 2003) confirmed that the additive contains a wide spectrum of mineral elements, the exact amounts of which were determined by atomic absorption analysis. No heavy metals were found to be present.

Biological research on the additive under greenhouse and field conditions showed that its application to the soil had both positive and negative effects on quantitative growth and yield parameters in the tested crops, depending on the dose, soil type and crop species.

The additive only had a positive influence on seed germination in bean and maize, where specific differences were observed in its utilization as a source of nutrients. Significant effects of the additive on the fresh and dry mass of maize and bean were recorded under greenhouse conditions when pre-grown plants with a sufficiently developed root system were planted. The good quality of the root system in maize and bean has an effect on the intensive nutrient uptake of the plants and significantly increases quantitative growth parameters, as ascertained by Vaněk et al. (2003) in several crop species. Nitrogen probably had the greatest influence on the increase in fresh and dry mass of maize and bean. A higher level of nitrogen is generally regarded, according to Masoni et al. (1990), as a major factor in the quantity of biomass and yield of crops, being the essential element with the greatest effect on photosynthetic intensity, the content of chlorophyll pigments and the accumulation of organic matter (Nátr, 1997). The role of nitrogen and the relationship between all the biogenic elements present in the additive appears to have had the greatest impact on photosynthetic intensity and the accumulation of fresh and dry mass of maize and bean, which corresponds with the results of Sabo et al. (2002).

In soil with higher humus content (soil type I) the additive was found to have a positive effect at the highest dose (20 g kg^{-1}), while in soil with very low humus content (soil type II) a positive effect was determined at the lowest dose (1 g kg^{-1}). This is in agreement with the findings of Fecenko (2003), who stated that it is uneconomical to fertilize soils of lower fertility with high doses of fertilizers or soils of good fertility with lower ones. It is suggested that the positive effects of the additive on soil type I were due, among other factors, to higher soil microorganism activity coupled with higher humus content and the good greenhouse conditions. The carbon in the additive could be a suitable source of energy for the production of CO_2 and other substances by microorganisms. According to Marendiak et al. (1987) microorganisms are very important for the cycles of other biogenic elements. The present results showed that the quality and structure of the soil, which are a function of various physical, chemical and biological properties, have a considerable influence on the regulation of nutrient uptake by plants from the soil and the additive, as also indicated by the results of Bedrna (1988) and Pechová et al. (2003).

Acknowledgements

This study was carried out in cooperation with the Agency for the Support of Science and Technology (APVP) with financial support from the bilateral Slovak-US project No. 027/2001. The authors wish to thank L. Matušková and J. Vojtáš from the Research Institute of Soil Science and Conservation in Bratislava for the analysis of the additive and soil samples, and to Assoc. Prof. A. Fargašová for her comments on the manuscript.

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Acta Agronomica Hungarica, 53(3), pp. 251–259 (2005)

EFFECT OF LOCATION AND YEAR ON SOME AGRONOMICAL CHARACTERS OF MAIZE HYBRIDS

Z. HEGYI, T. SPITKÓ and J. PINTÉR

AGRICULTURAL RESEARCH INSTITUTE OF THE HUNGARIAN ACADEMY OF SCIENCES, MARTONVÁSÁR, HUNGARY

Received: 21 June, 2005; accepted: 19 September, 2005

Some agronomical characters of twelve single-cross maize hybrids were investigated at five different locations in Hungary over a three-year period. The characters examined were individual plant production (total mass of the ears on a single plant), thousand-kernel mass, number of kernel rows, ear length, number of kernels per row, shelling % and the assimilating leaf area above the main ear.

Among these yield components, the individual plant production, the ear length, the number of kernels per row and the grain-cob ratio (shelling %) were influenced to the greatest extent by the year, followed by the variety and the location. The greatest average yield was achieved by the tested hybrids at all five locations in 1997 (263 g/plant). The average yields in 1998 and 1999 were significantly lower (221 and 203 g/plant, respectively). The outstanding yields achieved in 1997 could be attributed to the favourable ecological conditions, which led to the development of secondary ears in Keszthely and Sopronhorpács. At the other three locations there was only one ear per plant, but these ears were longer than in the following years. The greatest year effect was recorded in Sopronhorpács, where the individual plant production amounted to 305 g/plant in 1997 and 238 g/plant in the worst year, 1999. In Gyöngyös conditions were very dry in all three years, so the year effect was least pronounced at this location (grand mean of 195 g/plant in 1997 and 201 g/plant in 1999). Stability analysis was carried out using the coefficient of variance for individual plant production. Hybrids Mv 3, Mv 5, Mv 9 and Mv 12 were found to have the best adaptability. The shelling % was not significantly influenced by the location; the grain-cob ratio is relatively stable for maize hybrids. A correlation was found between the individual plant production and the leaf area above the main ear (R^2=0.658). Hybrids with the largest leaf area above the main ear also had the greatest ear mass.

Key words: maize hybrid, location effect, year effect, yield, yield components, assimilating leaf area

Introduction

The behaviour of maize hybrids in response to production and ecological factors can be described in terms of their adaptability or yield reliability. Maize is produced under varying ecological conditions. Agroecological factors can be divided into two fundamental groups: climatic factors (temperature, light, precipitation, relative air humidity) and edaphic (soil-linked) factors (Villax, 1944). Maize has great adaptability, so the soil is not as important in determining yields as in other crops. Precipitation and temperature, however, are important production conditions. Under Hungarian conditions the quantity and distribution of rainfall during the vegetation period is unfavourable (Marton et al., 1999). In

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an analysis of the agrometeorological conditions required for maize production, Polerecky (1976) found that 155–180 mm rainfall was required in July and August if high yields were to be achieved. Berényi (1945) also considered that under Hungarian production conditions the most critical month as regards rainfall was July, when the crop has the greatest water requirements, but there is very little rainfall at most locations. According to this author, fluctuations in rainfall quantities in July have a greater influence on the grain yield than variations in the temperature. A correlation coefficient of 0.6–0.8 was found between maize yields and the rainfall sum in July.

A wise choice of hybrid is an important factor in making maize production profitable. Varieties capable of high yields in favourable years and satisfactory yields in dry years should be given preference. The yield stability of the hybrids can be estimated from the yield fluctuations over years and locations (Szél, 1998).

Values of the heritability index (h^2) of maize yield components were reported by Hallauer and Miranda (1981), who found that the number of kernel rows was the yield component least influenced by environmental conditions (h^2=0.57), while the yield (h^2=0.18), the thousand-kernel mass (h^2=0.28), the number of ears per plant (h^2=0.39) and the ear length (h^2=0.38) were controlled by a large number of genes and were thus more sensitive to changes in the environment. The most important of the yield components are the number of ears per plant, the number of kernel rows, the thousand-kernel mass and the grain-cob ratio, or shelling percentage. According to Kapás (1961) the year had the greatest influence on the number of ears per plant, but also exerted a substantial effect on the thousand-kernel mass. Averaged over locations and varieties, the year had no effect on the number of kernel rows or the grain-cob ratio. The present authors also found that the shelling % (grain-cob mass ratio) was a stable index of the hybrids, which was not modified by the location (Gyenesné-Hegyi, 2000; Gyenesné-Hegyi et al., 2002).

The influence of environmental factors on the harvest index was investigated by Vulchinkov et al. (1995), who found that the year had the greatest effect. Other authors (Horner et al., 1971; Kim, 1975) also reported that the grain yield and the thousand-kernel mass were influenced by the environment, being modified not only by the location but also by the year. Dornescu (1979) also found that the location and the year had a substantial influence on the manifestation of heterosis. Among the yield components, the ear length was studied by Pavlicic (1974) and was found to be more dependent on the temperature and the humidity than on the genotype. Salazar and Hallauer (1986) carried out mass selection for ear length. The subpopulation from which the longest ears were selected had the most sensitive response to environmental effects. In experiments conducted by Mathur et al. (1997) the relationship between the yield components (number of kernel rows, kernel number, ear length, thousand kernel mass) and the grain yield remained constant and was not influenced by the environment to the least extent. In studies on variability in the

yield components, Veneni (1974) reported that the kernel mass per ear was the most variable over years and varieties (CV=16.8–35.4%), followed by the ear length (CV=9.2–15.6%) and the ear diameter (CV=4.66–8.24%). Stability analysis on yield components (Gyenesné-Hegyi et al., 2002) revealed that the most variable traits were the individual ear mass production (CV=9.3–42.4%), the grain mass (CV=17.5–30.0%) and the thousand-kernel mass (CV=11.1–20.3%). Moderate variance was recorded for the length of the main ear (CV=7.1–13.3%), while the number of kernel rows proved to be the least variable, being relatively insensitive to the environment (CV=6.6–9.2%).

Materials and methods

Twelve hybrids (Mv 1–Mv 12) were produced from seven inbred maize lines in the nursery of the Agricultural Research Institute of the Hungarian Academy of Sciences in 1996. From 1997 to 1999 these hybrids were tested at five locations (Keszthely, Gönc, Gyöngyös, Sopronhorpács, Martonvásár) in a random block design with three replications. The locations were chosen to give a good representation of the ecological differences existing within Hungary. The hybrids were sown at a plant density of 68,880 plants/hectare. The experimental soil was chernozem in Martonvásár and Gyöngyös and brown forest soil in Keszthely, Gönc and Sopronhorpács.

Morphological traits (length and breadth of the leaf next to the ear) were measured in the field after flowering on 5 plants from each treatment, and the number of leaves above the main ear was counted. The assimilating leaf area above the main ear was calculated using the equation reported by Montgomery (1906). Individual plant production (mass of all the ears per plant), the thousand-kernel mass, number of kernel rows and length of the main ears, the number of kernels per row, and the shelling % (grain-cob ratio) were recorded under laboratory conditions.

The climatic data for the experimental years and the 30-year data for these parameters at the five locations were provided by the National Meteorological Service. An analysis of the weather data revealed that in 1997 the rainfall distribution during the vegetation period was far better than in the following two years, especially as regards the quantity of rainfall in July. In this same year the mean temperature data were also more favourable, with fewer very hot days than in later years. Although there was more rainfall during the vegetation period in 1998, the distribution was unfavourable and there was a high number of very hot days at all the locations (129 in all). In Gyöngyös, for example, there were 31 days when the temperature was above 30°C for a long period. The experimental areas were all stricken by drought in all three years. The quantity and distribution of rainfall in 1999 was similar to that in 1998, but the mean temperatures were even higher. In Gyöngyös the mean temperature in July was 22.9°C, 2.2°C higher than the 30-year average, while it was only 19.5°C in the coolest location, Gönc. In Martonvásár, the experiments were irrigated with a water quantity equivalent to 80–100 mm rainfall in all three years to relieve the symptoms of drought.

The data were statistically analysed using the three-factor ANOVA program Agrobase'99, while discriminant analysis was carried out using the program package SPSS 11.0 for Windows.

Results

Individual plant production (total mass of ears per plant)

The main treatment effects and the interactions were significant at the P=0.1% level for this character. The MQ values indicated that the year had the greatest effect on the individual plant production of the hybrids, followed by the variety, while the location had the least influence. The hybrids produced the

greatest individual plant yield in 1997, when the grand experimental mean was 263 g/plant, while this value was significantly lower in 1998 and 1999 (221 and 203 g/plant, respectively).

At the various locations, the greatest individual plant production was recorded in Sopronhorpács in 1997, with a grand experimental mean of 305 g/plant and an ear number/plant of 1.3 (Fig. 1). The grand mean was significantly lower in Keszthely (283 g/plant) and Martonvásár (274 g/plant), where the ear number/plant was 1.1 and 1.0, respectively. In Gönc the mean individual plant yield of the hybrids was 259 g/plant, while the lowest yield was recorded in Gyöngyös (195 g/plant), with a single ear per plant at both locations. In 1998 the best production conditions were again found in Sopronhorpács, where the grand experimental mean was 241 g/plant. In this year the plants at all the locations produced only one ear. The difference between the Keszthely and Martonvásár locations was not significant (237 and 235 g/plant), while the grand experimental mean was again the lowest in Gönc (209 g/plant) and Gyöngyös (182 g/plant).

In 1999 the weather was most favourable for the hybrids in Martonvásár, where the highest individual plant production of 245 g/plant was achieved, with significantly lower average yields in Keszthely (233 g/plant) and Sopronhorpács (201 g/plant).

In Gönc the average yields were only a little lower than in Sopronhorpács (197 g/plant), though the difference was significant. In 1999, as in the other years, the lowest yields were observed for hybrids grown in Gyöngyös, where the individual plant production averaged 138 g/plant.

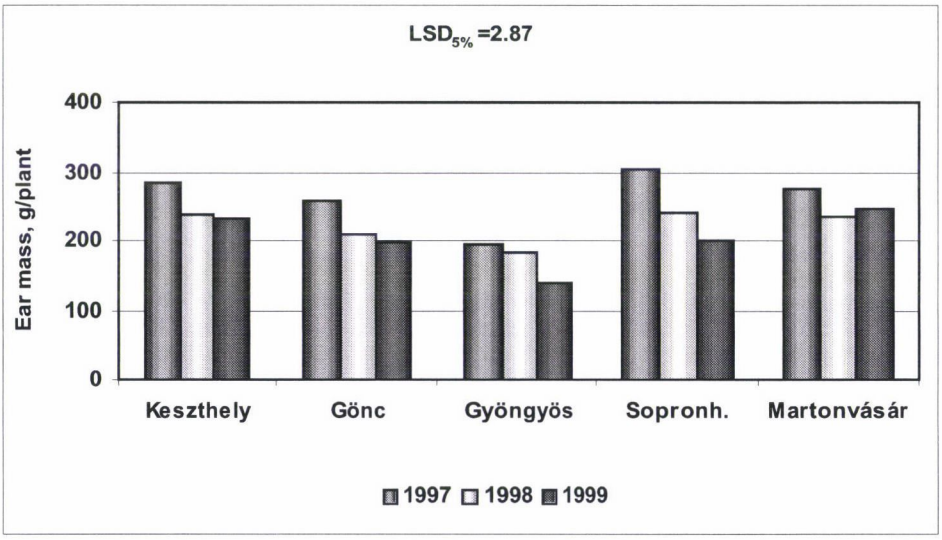

Fig. 1. Individual plant production recorded for years and locations, averaged over the hybrids (ear mass, g/plant)

The order of locations based on the individual plant production of the experimental hybrids was the same in 1997 and 1998 (Sopronhorpács, Keszthely, Martonvásár, Gönc, Gyöngyös), while in the third year (1999) it changed (Martonvásár, Keszthely, Sopronhorpács, Gönc, Gyöngyös).

In all three years the hybrids Mv 1, Mv 4 and Mv 8 gave the highest individual plant production. In these hybrids there was a great genetic distance between the parental components, while the individual plant production of hybrids developed from related lines (Mv 7, Mv 10, Mv 11) was smaller. The year had the greatest effect on the individual plant production of hybrids Mv 1, Mv 6, Mv 8 and Mv 9, which responded with high yields to the favourable ecological conditions in 1997, but gave yields far below their maximum yield potential in 1998 and 1999, though these values were still higher than the grand experimental mean (Fig. 2).

The stability of the hybrids at the various locations, averaged over the years, was analysed on the basis of the coefficient of variation (Table 1). The hybrids were divided into three groups on the basis of individual plant production, and those with small and large relative deviation (CV) were distinguished in each group. Among the seven hybrids that had individual plant yields in excess of the grand experimental mean (229 g/plant), Mv 3, Mv 4 and Mv 5 also had outstanding yield stability. These hybrids had ear masses above the grand experimental mean even in Gyöngyös, indicating their excellent adaptability. Hybrids Mv 1, Mv 6, Mv 8 and Mv 9 had high individual plant production values, but gave a sensitive response to extreme conditions (dry soil and air).

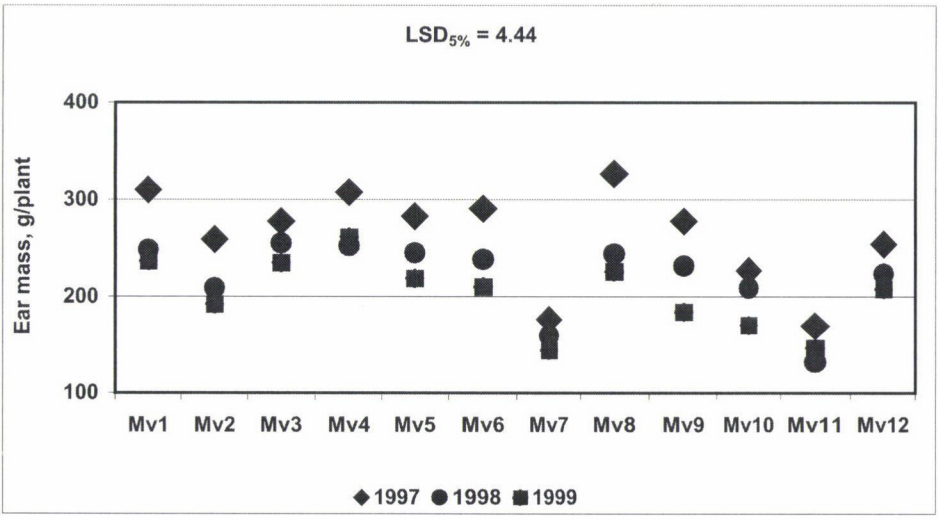

Fig. 2. Effect of year on the individual plant production of the hybrids, averaged over locations and hybrids (ear mass, g/plant)

Table 1
Stability of the hybrids in terms of the coefficient of variance (CV) for individual plant production (%)

200 g/plant		200–229 g/plant		229 g/plant <	
CV (6–14%)	CV (14% <)	CV (6–14%)	CV (14% <)	CV (6–14%)	CV (14% <)
Mv 7	Mv 10	Mv 12	Mv 2	Mv 3	Mv 1
Mv 11	–	–	–	Mv 4	Mv 6
–	–	–	–	Mv 5	Mv 8
–	–	–	–	–	Mv 9

The individual ear mass of hybrid Mv 1, for instance, averaged over the other factors, was 300 g/plant in Martonvásár, but only 212 g/plant in Gyöngyös. Hybrids Mv 2 and Mv 10 had moderately high yields (above the grand experimental mean), but also had high CV values. They were only capable of achieving their maximum yield potential under favourable conditions (Keszthely, Martonvásár), while they responded with yield losses to unfavourable environmental effects. The lowest yield level, combined with little relative deviation, was observed for two hybrids (Mv 7 and Mv 11). These hybrids, produced from related lines, proved extremely sensitive to environmental effects and produced yields below the grand experimental mean at all the locations.

Thousand-kernel mass

The results of analysis of variance (MQ values) demonstrate that, of all the factors analysed, the location had the greatest effect on thousand-kernel mass, followed by the variety and the year (in decreasing order). Averaged over three years, the thousand-kernel mass of the hybrids was greatest in the Keszthely trials (378 g), while the lowest values were recorded in Gyöngyös (301 g). The greatest difference between the locations was thus 77 g, compared with 20 g between the years. The hybrids with the greatest thousand-kernel masses (Mv 1, Mv 4, Mv 8) also produced the highest yields (378.9, 371.7 and 362.7 g, respectively), while the smallest thousand-kernel mass (239.3 g) was recorded for the only flint hybrid (Mv 11), which had the lowest individual plant production.

Number of kernel rows

Among the factors tested, the variety had the greatest influence on the number of kernel rows, followed by the year, while the location had the least effect. Among the yield components the number of kernel rows had the highest value of h^2 (0.57), indicative of the substantial genetic determination for this trait and the slight effect of the environment. The Mv 11 hybrid, the only flint hybrid tested, had the largest number of kernel rows (17.2), while the smallest number was counted for Mv 1 (14.4) and Mv 10 (14.6).

Averaged over all the factors, the number of kernel rows did not differ significantly in 1997 and 1998 (16.2 and 16.1, respectively), while in 1999 all the hybrids had a smaller number of kernel rows (15.8, averaged over all the factors). This value was significantly lower than in the other two years.

Ear length and number of kernels per row

The MQ values demonstrated that of all the factors tested the year had the greatest effect on the ear length and number of kernels per row, followed by the variety and the location. The longest ears were found in 1997 (20.2 cm), while the main ears were significantly shorter in 1998 (19.1 cm) and 1999 (17.6 cm). The greatest year fluctuation for this parameter was recorded in Gyöngyös, where there was a difference of 5 cm between the length of the main ear in the best (1997) and worst (1999) years. The smallest difference between these same years was recorded in Martonvásár (0.3 cm).

The value of the Bravais correlation coefficient between ear length and the number of kernels per row was 0.74. In Gyöngyös the ear tips were not fertilised due to the drought, and this reduced the closeness of the correlation.

Grain-cob ratio (shelling %)

The MQ values calculated by analysis of variance indicated that the grain mass and cob mass were both influenced to the greatest extent by the year, followed by the variety and the location, but even this effect was not great. The grain-cob ratio was 84:16% averaged over all the factors in 1997, while this value was 85:15% in 1998 and 86:14% in 1999. The location had no significant effect on the ratio, the shelling percentage being 85:15% at all the locations.

Hybrids Mv 4, Mv 6, Mv 8 and Mv 12 had the best shelling percentages (85:15%), while the lowest values were recorded for hybrid Mv 7, where the grain-cob ratio was 81:19%.

Correlation between the assimilating leaf area above the main ear and the individual plant production

The MQ values calculated by analysis of variance indicated that the assimilating leaf area was influenced to the greatest extent by the year, followed by the variety and the location. This parameter had the highest value in 1997 (4267 cm^2) and was significantly lower in 1998 (4088 cm^2) and 1999 (3805 cm^2). The values recorded for the different genotypes ranged from 3249 to 4871 cm^2. A moderately strong correlation was observed between the assimilating leaf area above the main ear and the individual plant production, the value of Pearson's correlation coefficient being 0.66 (Fig. 3).

The data were also analysed using discriminant analysis to determine the extent to which the tested parameters were able to discriminate between the locations. The program compared pairs of locations and calculated the values of the F test, indicative of the differences between the locations.

The analysis indicated that the Gönc and Gyöngyös locations differed from each other to the greatest extent, as the value of the coefficient was greatest for this pair (38.210). The closest correlations were obtained between Sopronhorpács and Keszthely (10.335) and between Martonvásár and Keszthely (9.911).

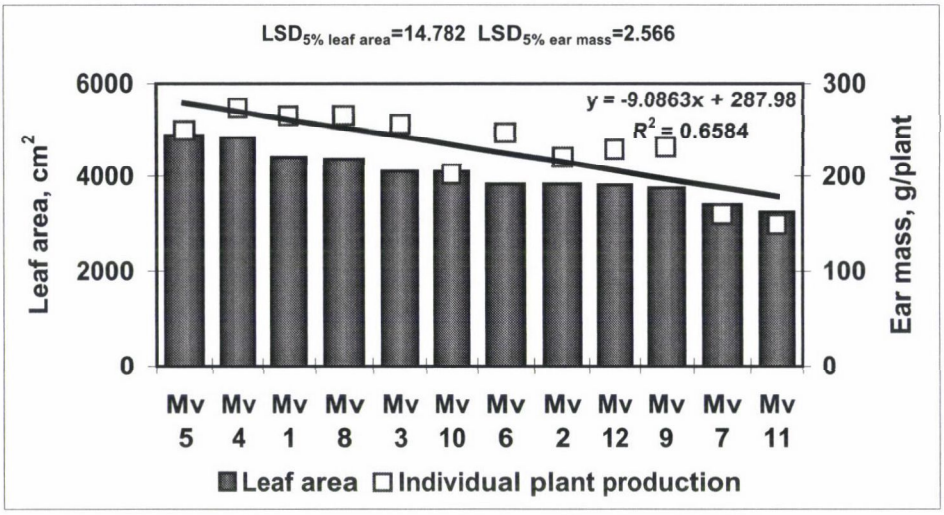

Fig. 3. Ranking of hybrids based on the correlation between assimilating leaf area and individual plant production, averaged over all the factors (cm^2, g/plant)

Discussion

The experimental results confirmed those of Berényi (1945) and Polerecky (1976), who found that the quantity of rainfall in July had a decisive effect on the maize yield. The individual plant production of the hybrids was greatest at locations where the rainfall quantity in July reached the average over many years (Keszthely, Sopronhorpács) or where irrigation was carried out during flowering (Martonvásár). The location and the year both modified the frequency with which secondary ears were formed, though the effect of the year was greater than that of the location (Kapás, 1961). In agreement with the results published by Kapás (1961), Lucas and Remison (1984) and Gyenesné-Hegyi et al. (2002), the data demonstrated that the grain-cob mass ratio of the hybrids was a stable trait, not influenced by the location. Among the yield components, the most variable were the ear mass, the thousand-kernel mass, the ear length and the number of kernels per row, in agreement with other authors (Horner et al., 1971; Kim, 1975; Dornescu, 1979; Pavlicic, 1974). The most constant yield component was the number of kernel rows, as indicated by Hallauer and Miranda (1981). The year had the greatest effect on the assimilating leaf area, confirming the results of Duncan (1975), who stated that the environmental effects experienced during leaf development had an influence on the size of the leaf blades. The leaves with the largest leaf area were grown in Keszthely and the smallest in Gönc, probably due to the cooler weather in the latter. The value of Pearson's correlation coefficient between the individual plant production and the assimilating leaf area above the main ear was high (0.88), as also reported by Seka and Cross (1995) when investigating the effect on plant size of the genes responsible for grain mass.

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Acta Agronomica Hungarica, 53(3), pp. 261–272 (2005)

GENEALOGICAL ANALYSIS OF DIVERSITY OF SPRING BREAD WHEAT CULTIVARS RELEASED IN KAZAKHSTAN FROM 1929–2004

S. P. MARTYNOV[1], T. V. DOBROTVORSKAYA[1], A. I. MORGOUNOV[2], R. A. URAZALIEV[3] and A. S. ABSATTAROVA[3]

[1]N. I. VAVILOV RESEARCH INSTITUTE OF PLANT INDUSTRY (VIR), ST. PETERSBURG, RUSSIA
[2]CIMMYT, ALMATY, KAZAKHSTAN [3]RESEARCH CENTRE OF AGRICULTURE AND CROP PRODUCTION, ALMALYBAK, KAZAKHSTAN

Received: 5 April, 2005; accepted: 12 September, 2005

The genetic diversity of 116 spring bread wheat cultivars released in Kazakhstan from 1929–2004 was studied by means of a genealogical analysis. The tendency of genetic diversity to change over time was traced by analysing a series of $n \times m$ matrices, where n is the number of released cultivars and m is the number of landrace ancestors. The pool of landrace ancestors of spring wheat cultivars in 1929–2004 contained a total of 114 landraces and old varieties, including 19 from Kazakhstan and Central Asia and 23 from neighbouring regions of Russia. The original ancestors differ significantly in frequency of presence and hence in their importance in the genepool of spring wheats cultivated in Kazakhstan. Significant differences in the contributions of dominant ancestors to cultivars for various regions have been revealed, showing that those ancestors were specifically adapted to different growing conditions. During the past 75 years, genetic diversity has increased due to the wide use of foreign materials in breeding programmes. A more detailed study has shown that during the period analysed, 15 landraces from Kazakhstan and neighbouring regions of Central Asia and Russia (35% of local germplasm) were lost from the pedigrees. The cluster structure of modern cultivars included in the Kazakhstan Official List (2002) was established. By analysing coefficients of parentage, significant differences in the genetic diversity of cultivars from various growing regions were revealed.

Key words: bread wheat, diversity, pedigree, adaptation, landraces

Introduction

Kazakhstan is one of the world's major grain producers and exporters. Annually Kazakhstan produces 15–18 million tons of wheat, of which 7–8 million tons are used for domestic consumption and about 5.5 million tons are exported (Makus, 2003; Urazaliev, 2003). Spring wheat is the main export grain crop in Kazakhstan. It is cultivated basically in Northern Kazakhstan and occupies about 10 million ha. The favourable climatic conditions in Kazakhstan promote the production of superior quality, high-protein grain, which is in great demand on the world food market as a wheat flour quality enhancer.

Cultivars developed in Kazakhstan are cultivated on 3–4 million ha in the country and on 2.5 million ha abroad. There are 54 spring wheat cultivars included in the Kazakhstan Official List of Released Varieties, including 25 developed by breeders in Kazakhstan (Esimov, 2004). It is known that the level

0238–0161/$ 20.00

of latent genetic diversity in a crop affects its vulnerability to diseases and pests, and determines its stability despite environmental stresses. Therefore, analysing changes in the genetic diversity of a set of cultivars recommended for cultivation is useful both for guiding the breeding programmes and for establishing a production strategy.

When the genealogical approach is used, the measures of diversity are coefficients of parentage for all pairwise combinations of cultivars, the structure of genetic profiles, average contributions of major landrace ancestors, and the frequency of their presence in the pedigrees of a given set of cultivars. The tendencies towards temporal or spatial changes in genetic diversity are revealed by the separation and analysis of sets of cultivars released in different periods of time or growing in different regions.

The objective of this study was to quantify the latent genetic diversity of spring bread wheat cultivars released in Kazakhstan and to analyse its tendency towards change over time and space.

Materials and methods

The pedigrees of 116 spring wheat (*Triticum aestivum* L.) cultivars released in Kazakhstan between 1929 and 2004 were the subject of this study. The cultivar Erythrospermum 35 (Erythrospermum 29-70-19/Lutescens 26-67-211) was excluded from the analysis because the pedigrees of the parents are unknown. Pedigree analysis and the calculation of the coefficients of parentage matrix were carried out with the aid of the Information and Analytical System of Wheat Genetic Resources GRIS3.5 (Martynov and Dobrotvorskaya, 2000). The change in diversity over time was estimated based on genetic profiles (Martynov, 1998). The diversity of modern cultivars included in the Kazakhstan Official List was studied using cluster analysis on the cultivar × cultivar matrix of coefficients of parentage (algorithm UPGMA). Cluster analysis was conducted using the software package NTSYS 2.02c (Rohlf, 1998).

Results and discussion

It is natural to assume that the original ancestors of a set of cultivars characterize their genetic diversity. The genetic profiles of 115 cultivars were constructed to analyse changes in the diversity of the spring wheat cultivars released in Kazakhstan from 1929 to 2004. The pool of original ancestors for the entire period included 114 landraces and old varieties, as well as 14 parents of unknown origin. Between 42 and 37% of all landraces were from Kazakhstan or from neighbouring regions of Central Asia and Russia. The same number of landraces (42) was from European countries. The number of ancestors from other continents was much lower: Asia – 9; the Americas – 11; Africa – 10. The original ancestors differ greatly in frequency of presence in the analysed cultivars and, hence, in their importance in the genepool of spring wheat cultivars released in Kazakhstan. Such landraces as Poltavka, Selivanovsky Rusak, Beloturka, Hard Red Calcutta, Ostka Galicyjska, Crimean and Mediterranean are present in the pedigrees of most cultivars, with a frequency of

61 to 82%. Other landraces (Kozhe-biday, LV-Krasnodar via Lutescens 47) dominate only in varieties cultivated in southern regions. About half of the original ancestors (51) is present in the pedigrees of only 1 or 2 cultivars (frequency 0.9–1.7%). The ancestor average contribution is the average coefficient of parentage for all cultivars. It estimates the contribution of an ancestor to a cultivar's pedigree and varies from 0.178 for Poltavka to less than 0.00001 for Ble Seigle, Ghirka Spring, and others.

A two-way analysis of variance for a randomized design was used to analyse the original ancestors' specific distribution in different regions of the country. The administrative regions (oblasts) of Kazakhstan have been organized into five agricultural regions differing in natural climate conditions (precipitation and temperature) and belonging to different production environments. The Northern region is made up of four oblasts: Akmola, Kostanay, Pavlodar and North Kazakhstan. This production environment belongs mainly to the Kazakhstan steppes (code 6-5) and the dry steppes (code 7-4). Drought and average temperatures are characteristic of the region. The Southern region includes the Almaty, Zhambyl, Kyzyl-Orda and South-Kazakhstan oblasts and is referred to as the Aral-Balkhash desert (9-2), the South-Kazakhstan foothill desert-steppes (10-1) and the Central Asian foothill semi-desert (12-1) regions. All three environments are classified as dry with high temperatures. The Eastern region includes the East Kazakhstan oblast and is classified as the Altay foothill steppes (6-6) and the Kazakhstan dry steppes (7-4); both environments are considered dry or very dry, with lower than average or average temperatures. The Western region includes the Aktyube, Atyrau and West Kazakhstan oblasts, now classified as the Trans-Volga steppes (6-4), the dry steppes (7-3) and the Caspian semi-desert (8-1) environments, which are very dry with average or above-average temperatures. The Central region includes Karaganda oblast belonging to the Kazakhstan dry steppe environment (7-4) (Natural and Agricultural Zones of the USSR, 1975).

Regions (factor *A*) with gradation number $a=5$ and dominant original ancestors (factor *B*) with gradation number $b=13$ were the factors in the analysis of variance. The following dominant original ancestors with 50% frequency or higher in one or more regions were included in the calculations: Poltavka, Selivanovsky Rusak, Beloturka (from the Saratov district of Russia), Kozhe-Biday, LV (local variety) from Krasnodar district (via Kazakhstanskaya 126), Banatka, Crimean (UKR), Ostka Galicijska (POL), Hard Red Calcutta (IND), Mediterranean (Europe), Akakomugi (JPN), Rieti (ITA) and Zeeuwse (NLD). The effects of the two factors and their interactions were significant (Table 1). The significant interaction $(A \times B)$ shows a difference in the distribution of dominant ancestor contributions to cultivars of various regions and, hence, in the specific adaptation of original ancestors to different cropping conditions (Table 2).

Table 1
Two-way analysis of variance of the contributions of dominant original ancestors of modern spring wheat cultivars over regions of Kazakhstan (data subjected to arc-sine transformation)

Source	S.S.	D.F.	M.S.	F
Total	76458.3	870		
Regions (factor *A*)	669.1	4	167.27	3.64*
Ancestors (factor *B*)	29746.7	12	2478.89	53.96**
Interaction (*A*×*B*)	9017.8	48	187.87	4.09**
Error	37024.7	806	45.94	

* Significant at the $P<0.05$ level; ** Significant at the $P<0.0001$ level

Table 2
Contributions of dominant ancestors to spring wheat cultivars in different regions of Kazakhstan

Landraces	Regions				
	North	South	East	West	Central
Poltavka	0.185*b*	0.104*a*	0.278*c*	0.323*c*	0.359*c*
Selivanovsky Rusak	0.050*ab*	0.036*a*	0.065*ab*	0.050*a*	0.074*b*
Beloturka (*T. durum*)	0.025*ab*	0.016*a*	0.034*ab*	0.037*ab*	0.057*b*
Banatka (Ukraine)	0.034*b*	0.017*ab*	0.016*ab*	0.000*a*	0.009*a*
Kozhe Bidaj	0.000*a*	0.105*b*	0.000*a*	0.000*a*	0.000*a*
LV-Krasnodar	0.000*a*	0.079*b*	0.000*a*	0.000*a*	0.000*a*
Ostka Galicyjska	0.027*a*	0.011*a*	0.029*a*	0.006*a*	0.006*a*
Hard Red Calcutta	0.028*ab*	0.005*a*	0.034*b*	0.008*a*	0.008*ab*
Crimean	0.034*bc*	0.035*c*	0.028*abc*	0.007*a*	0.004*a*
Mediterranean	0.010*a*	0.086*b*	0.012*a*	0.013*a*	0.000*a*
Akakomugi	0.008*a*	0.007*a*	0.004*a*	0.000*a*	0.000*a*
Rieti	0.004*a*	0.004*a*	0.002*a*	0.000*a*	0.000*a*
Zeeuwse	0.001*a*	0.001*a*	0.001*a*	0.001*a*	0.000*a*

Values within the same column followed by a different letter are significantly different at the 0.05 probability level by Duncan's multiple range test (comparisons made after arc-sine transformation)

The landrace Poltavka has the highest contribution on average across regions, showing its broad adaptation, which is mainly due to its ability to tolerate abiotic stresses (Flaksberger, 1935). As shown in Table 2, Poltavka made the maximum contribution in the Western, Central and Eastern regions. The high frequency of presence of landraces Selivanovsky Rusak and Beloturka in the Northern (84%), Central (86%) and Eastern (75%) regions is explained by the wide use in crosses of Saratovskaya 29, whose pedigree includes Poltavka, Selivanovsky Rusak and Beloturka. Selivanovsky Rusak and Beloturka confer excellent drought tolerance and good grain quality to their descendants. Among the modern spring wheat varieties released in Kazakhstan, 76.5% are the descendants of Saratovskaya 29, which is still cultivated in some areas of the country.

The Kazakhstan landrace Kozhe-Biday (*T. compactum* L.) was released in the Akmola, East Kazakhstan and Karaganda districts between 1938 and 1958. Kozhe-Biday and LV-Krasnodar are present in the pedigrees of half the cultivars recommended for the Southern region via variety Kazakhstanskaya 126, a superior quality wheat developed at the Kazakh Research Institute of Agriculture by the outstanding breeder N. L. Udolskaya. This cultivar was released in 1955–1984 in Almaty oblast and in Kyrgyzstan. Landrace Kozhe-Biday made a high contribution to the pedigree of cultivars for the Southern region, but is not present in the pedigrees of cultivars in other regions of Kazakhstan.

The local Ukrainian winter wheat variety Banatka participates in the pedigrees of spring wheats due to crosses between spring and winter parents. This old variety has the highest contribution in the West Siberian region of Russia, adjacent to Northern Kazakhstan. Banatka has the highest frequency (74.2%) in cultivars released in North Kazakhstan. This is probably due to the wide distribution of cultivars developed with the participation of winter wheat in this region, for example, Omskaya 18, 19, 20, 24, 29, 30, and Pamyati Azieva from Western Siberia and Kazakhstanskaya 15, 19, 25, etc., developed in Kazakhstan.

Ostka Galicijska and Hard Red Calcutta appeared in the pedigrees of most cultivars (especially in the Northern and Eastern regions) due to hybridization with the Canadian cultivar Marquis. The landraces Mediterranean and Crimean have made significantly higher contributions to the pedigree of cultivars in the Southern region. The Japanese landrace Akakomugi, the Italian Rieti and the Dutch Zeeuwse are present in the pedigrees of half of all cultivars in the Northern and Southern regions. However, their contributions are very small, and the differences in contributions between regions are not significant. These landraces are also included in the pedigrees of spring wheat cultivars due to hybridization with winter wheats.

The tendency of diversity to change over time can be traced by analysing a series of $n \times m$ matrices, where n = number of released cultivars and m = number of original ancestors (landraces). Genetic profiles were constructed for all cultivars released in Kazakhstan from 1929 to 2003 and 75 matrices were generated to analyse the change in diversity over time. This made it possible to estimate changes in the structure of original ancestors in the cultivars released each year (Fig. 1). As has been noted, the pool of original ancestors of spring wheats cultivated in Kazakhstan over the entire period included a total of 114 landraces plus 14 lines of unknown pedigree. Although landrace accumulation in the pedigrees (top curve) was accompanied by the loss of several of them (bottom curve), the actual number of landraces in the pedigrees increased. During the analysed period, the total number of landraces participating in the pedigrees of released cultivars increased from 4–14 in 1930 to 94–99 in 2000.

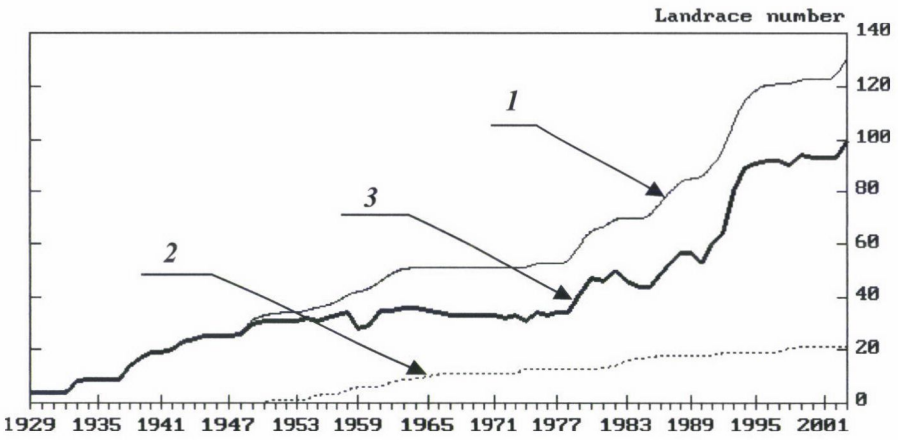

Fig. 1. Change in the total number of landraces contained in the pedigrees of spring wheat cultivars released in Kazakhstan from 1929 to 2003. 1=accumulation of landraces in the pedigrees; 2=total number of lost landraces; 3=actual number of landraces in the pedigrees over time

The number of spring wheat cultivars released over the years (Fig. 2) shows an increase in the first 30 years, from 5 in the early 1930s to 29–31 in the late 1950s. The number of cultivars then began to decrease, reaching a minimum (15–17) in the 1970s. During the last 30 years the number of released cultivars has increased to 39–44 in 2000–2004. The genetic diversity of the cultivars released during the 75-year period gradually expanded, as demonstrated by the average number of landraces per pedigree. Up to the mid-1960s, the pedigrees contained only 1–3 landraces per variety, but have become more complex since the 1970s, as the number of original ancestors per cultivar increased. The genetic profiles of cultivars recently released in Kazakhstan (2000–2004) include an average of 14–16 landraces per variety (Fig. 2). The more sophisticated pedigrees in modern varieties are evident in the curve of increase in the average number of ancestors (i.e. all accessions involved in a cross) per cultivar. The pedigrees contained an average of 4–8 ancestors in the early 1930s. In subsequent years, the pedigrees of newly released cultivars became more complex: the average number of ancestors in the early 1940s was 19–23 and 31–34 in the 1970s. Since the late 1980s, the rate of increase in the number of ancestors has exceeded the rate of expansion in genetic diversity, estimated based on the increase in the average number of landraces per pedigree (Fig. 2).

Thus, in the last 75 years, genetic diversity has increased. The number of landraces from different regions participating in the pedigrees of cultivars released in Kazakhstan (Fig. 3) shows that the increase in genetic diversity in the 1980s and especially in the 1990s was primarily due to hybridization with foreign materials, mainly from North America, for example, FKN-25, Gaines,

Justin, Lee (USA), Mexipak, Nadadores 63, Pitic 62 and PV-18 (CIMMYT). However, the analysis has also shown that during that same period, 15 landraces, or 35% of all ancestors, were lost from the pedigrees (Table 3). It may be assumed that many of them, though not as productive as modern cultivars, may have carried a complex of genes conferring specific adaptation to conditions in Kazakhstan. Thus, the substantial growth in genetic diversity was accompanied by the genetic erosion of local materials.

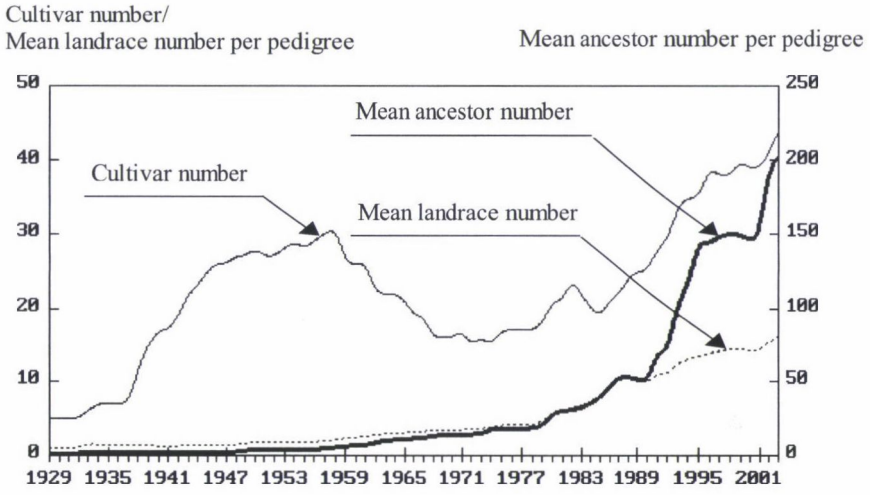

Fig. 2. Average number of ancestors, average number of landraces per pedigree, and number of spring wheat cultivars released in Kazakhstan over time

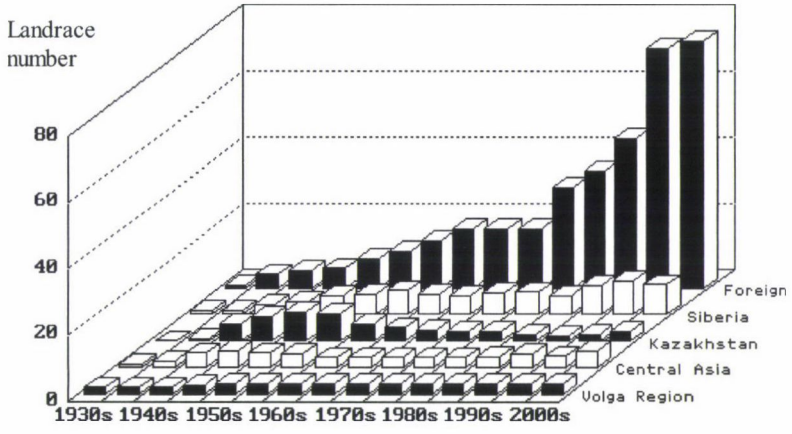

Fig. 3. Number of landraces from various areas participating in the pedigrees of spring wheat cultivars released in Kazakhstan from 1929 to 2004

Table 3
Original ancestors of spring wheat cultivars lost over time

Original ancestor	Year
Kitaiskaya alaya (Chinese Scarlet)	1951
LV-Karshi district of Uzbekistan (via Graecum 283)	1955
Graecum Chingirlau	1955
LV-Karshi district of Uzbekistan (via Graecum 289)	1958
LV-Katta-Kurgan district of Uzbekistan (via Erythrospermum 5755)	1958
Ak Biday	1959
Kara Keltek	1962
LV-Tara raion of Omsk district (via Albidum 3700)	1962
Syr Biday	1963
Red ear wheat from Omsk district of Russia	1965
LV-Dzhuvali raion of Zhambyl district	1966
LV-Kostanay (via Ferrugineum 1458)	1974
Kubanka	1974
Red ear wheat from Altay district of Russia	1983
Sandomirka	1986
LV-Ust-Kamenogors district (K-34291)	1992

A matrix of the coefficients of parentage for all pairwise combinations of the 51 cultivars was computed on the basis of their pedigrees to estimate the genetic diversity of modern spring wheats included in the Kazakhstan Official List. Cluster analysis (algorithm UPGMA) was carried out in two ways: (1) the matrix of coefficients of parentage was considered as a matrix of similarity measures; and (2) the correlation coefficient between cultivars for parentage coefficients was used as an index of similarity. This means that the matrix of coefficients of parentage was considered as the data matrix, and the index of similarity was calculated as coefficients of correlation between coefficient of parentage vectors for the compared pairs of cultivars. Although the results of both cluster analyses coincided for the most part, with the second method the dendrogram produced was more distinct (Fig. 4).

The set of cultivars included in the Kazakhstan Official List in 2002–2004 has a cluster structure. Among 51 cultivars at the half-sib level, six clusters were revealed. The largest cluster (*B*) contains more than half (28) of all the cultivars, while five smaller clusters (*A, C, D, E, F*) contain 2 to 5 cultivars. The average within- and between-cluster coefficients of parentage are given in a triangular matrix (Table 4). The average weighted value of the coefficient of parentage within clusters is 0.225. Within-cluster coefficients of parentage (diagonal) are much more appropriate than between-cluster coefficients; therefore, the results of cluster analyses can be considered statistically proved. The revealed clusters explain 92% of the genetic diversity of modern spring wheat cultivars included in the Kazakhstan Official List in 2002–2004.

Pair cluster *A* includes cultivars developed at Krasnyj Kut Breeding Station: Erythrospermum 841 and its descendant Albidum 28.

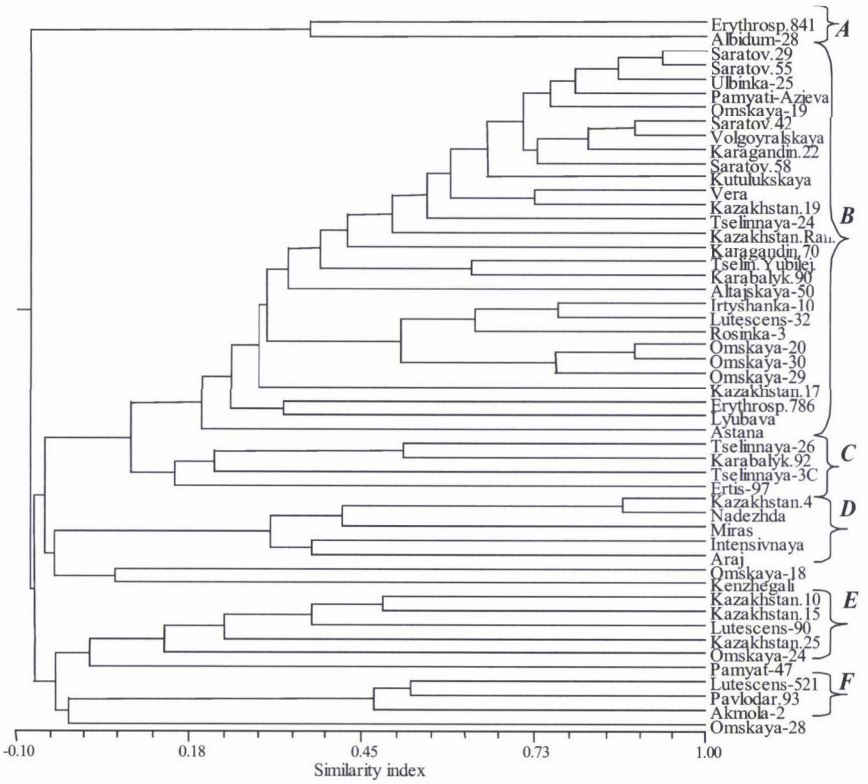

Fig. 4. Results of cluster analysis based on coefficients of parentage among 51 spring wheat cultivars included in the Kazakhstan Official List in 2002–2004

Table 4

Within- (diagonal) and between-cluster coefficients of parentage among spring wheat cultivars included in the Kazakhstan Official List in 2002–2004

Cluster*	*A*	*B*	*C*	*D*	*E*	*F*
A (2)	0.224	0.032	0.007	0.012	0.005	0.007
B (28)		0.224	0.064	0.102	0.083	0.044
C (5)			0.259	0.029	0.050	0.007
D (4)				0.172	0.051	0.056
E (5)					0.203	0.042
F (3)						0.279

* The number of cultivars in a cluster is specified in brackets

Cluster *B* contains 28 cultivars derived from either variety Saratovskaya 29 or its full sibs Saratovskaya 36 and Saratovskaya 39. The average contribution of cultivar Saratovskaya 29 to this cluster is mc=0.40, which is equivalent to a relationship at a level between full (0.5) and half-sibs (0.25). The pedigrees of all cultivars in this numerous cluster contain Poltavka, a local variety from the Saratov region, whose average contribution is very high

(*mc*=0.31). All cultivars developed by the Saratov breeding institute (Saratovskaya 29, 42, 55, 58, Volgouralskaya and Erythrospermum 786), several developed by the Kazakhstan breeding institute (Astana, Kazakhstanskaya 17, Kazakhstanskaya 19, Kazakhstanskaya rannespelaya, Karagandinskaya 22, Karagandinskaya 70, Karabalykskaya 90, Lyubava, Lutescens 32, Ulbinka 25, Tselinnaya 24 and Tselinnaya yubileinaya), and several from other breeding institutes, mainly in Western Siberia (Altayskaya 50, Vera, Irtyshanka 10, Kutulukskaya, Omskskaya 19, 20, 29, 30, Pamyati Azieva and Rosinka 3) are grouped together in cluster *B*.

Cluster *C* includes five Kazakhstan cultivars (Araj, Intensivnaya, Kazakhstanskaya 4, Miras and Nadezhda) derived from Kazakhstanskaya 126; cultivars in this cluster have an average coefficient of parentage of 0.42.

Cluster *D* contains cultivars derived from Kazakhstan cultivar Shortandinka 25: Ertis 97, Karabalykskaya 92 and Tselinnaya 26 and 3C. Shortandinka 25's average contribution to this cluster is *mc=0.37*.

All cultivars in cluster *E* (Kazakhstanskaya 10, 15, 25, Lutescens 90 and Omskaya 2) have winter wheat cultivar Bezostaya 1 in their pedigrees, with an average coefficient of parentage of 0.34. Winter wheat cultivar Mironovskaya 808 appears in the pedigrees of all cultivars in cluster *F* (Akmola 2, Lutescens 521 and Pavlodarskaya 93), with an average coefficient of parentage of 0.54. Four cultivars did not cluster at all: Kenzhegali, Omskaya 18 and 28, and Pamyat 47. Information on the pedigrees of two of them (Pamyat 47 and Omskaya 28) is incomplete.

The coefficients of parentage between all pairwise cultivar combinations vary from 0 to 0.684, with an average R=0.108. This value indicates there is sufficiently high diversity in modern spring wheat cultivars released in Kazakhstan. Nevertheless, it is interesting to compare the diversity of cultivars recommended for various regions. The genetic diversity per wheat-producing region is estimated based on the coefficients of parentage for all possible combinations within cultivar groups for each region. The coefficients of parentage between cultivars recommended for cultivation in five regions have been subjected to analysis of variance (Table 5). The results reveal significant differences (P<0.001) in the genetic diversity of cultivars recommended for different regions of Kazakhstan.

Table 5

Analysis of variance of coefficients of parentage between spring wheat cultivars included in the Kazakhstan Official List in 2002–2004 based on growing regions

Source	S.S.	D.F.	M.S.	F
Total	10.33	617		
Regions	0.44	4	0.1090	6.76*
Error	9.89	613	0.0161	

* Significant at the P<0.001 level

As is well known, the threshold for a safe level of cultivar similarity is the coefficient of parentage between quarter-sibs (0.125). According to this estimate, the genetic diversity of cultivars recommended for the Northern, Southern, Eastern and Western regions of Kazakhstan is sufficiently high. Only cultivars for the Karaganda region (Central region) have an average coefficient of parentage of 0.23, which is close to the relationship between half-sibs. This limited diversity could have dangerous consequences because it increases the likelihood of severe disease epidemics occurring in the region. A narrow genetic base can also increase a crop's vulnerability to other biotic and abiotic stresses (Table 6).

Table 6
Average coefficients of parentage of spring wheat cultivars released in different wheat-producing regions of Kazakhstan

Region	Cultivar number*	Average coefficient of parentage**
North	31	0.11*ab*
South	10	0.09*a*
East	12	0.16*b*
West	7	0.14*ab*
Central	7	0.23*c*
All Kazakhstan	51	0.11

* Some cultivars are recommended for several regions; therefore, the total number of cultivars is higher than the number included in the Official List; ** Average coefficients of parentage followed by a different letter are significantly different at the 0.05 probability level by Duncan's multiple range test

Conclusions

The genealogical analysis of the genetic diversity over space and time of the set of spring bread wheat cultivars released in Kazakhstan over a 75-year period has been investigated. Significant differences were found in the contributions of major original ancestors to cultivars released in different regions and, hence, the specific adaptation of progenitor landraces to various cropping conditions has been established. The pool of original ancestors gradually increased over the years, as did the rate of expansion of genetic diversity over the last 30 years. Nevertheless, the increase in diversity was accompanied by the loss of local original ancestors, estimated at approximately 35%. The replacement of local germplasm by more productive foreign materials could have undesirable consequences, because many of the lost landraces carried a complex of genes controlling adaptation to specific conditions in Kazakhstan. From this perspective, the replacement process could be viewed as genetically eroding the released diversity.

The set of cultivars included in the Kazakhstan Official List in 2002–2004 has a cluster structure. The clusters explain 92% of the cultivars' genetic diversity. More than half of these cultivars belong to a large cluster containing

derivatives of the variety Saratovskaya 29. Smaller clusters grouped based on their ancestry were classified as derivatives of Kazakhstanskaya 126, Shortandinka 25, Bezostaya 1 and Mironovskaya 808.

In conclusion, high levels of genetic diversity were observed in most spring wheat-producing regions, except for cultivars recommended for the Central region, whose level of similarity could be viewed as near critical.

Acknowledgements

The authors are grateful to M. A. Berdagulov, Head of Department (Karabalyk Agricultural Experiment Station); Y. I. Zelenskiy, Head of Department (A. I. Baraev Research Centre of Grain Production), and G. A. Sereda, Head of Department (Agricultural Research Institute for Central Kazakhstan) for providing the pedigrees of Kazakhstan cultivars.

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Acta Agronomica Hungarica, 53(3), pp. 273–282 (2005)

EFFECTS OF PRECURSOR CROPS AND MANAGEMENT LEVELS ON THE STRAW AND GRAIN YIELD OF WHEAT AT HORRO HIGHLAND, WESTERN OROMIYA

T. ABERA and M. BELISSA

OROMIYA AGRICULTURAL RESEARCH INSTITUTE, BAKO AGRICULTURAL RESEARCH CENTER, BAKO, WESTERN OROMIYA, ETHIOPIA

Received: 17 July, 2005; accepted: 19 August, 2005

The sustainable production of wheat may be possible by integrating crop rotation with improved crop management practices. The maximum grain yield of wheat was observed when field pea was the precursor crop. The precursor crop and management levels showed a significant effect on the mean straw and grain yields of wheat. Field pea as precursor crop gave a better wheat grain yield with both improved and farmers' cultural practices. Both local and improved varieties gave a better response to management levels on the field pea precursor field. Local and improved varieties gave higher yields with intensive management and chemical fertilizer application. Field pea as precursor crop gave a combined grain yield advantage of 32% relative to barley. Management practices produced a combined grain yield advantage of 16 to 73% when field pea was the precursor crop, compared to barley. The use of field pea as precursor crop with improved management practices is essential to maximize wheat yields. Better grain yields and higher net returns were achieved with field pea as precursor crop compared to barley. Using field pea as precursor crop is the most successful management option for sustainable wheat production.

Key words: precursor crops, wheat, management level, Horro

Introduction

The dynamic changes in biotic and abiotic factors determine the long-term production and productivity of agriculture (Tanner et al., 1999). Human interference with existing natural processes is very swift, but intervention to amend deterioraton is very slow. The design of crop production must consider the arrangement of crops in time and space, the surrounding environment, and the type and intensity of management practices applied (Altieri, 1999). the production of wheat may be limited by a lack of the appropriate cropping system and crop management practices. Wheat yields were 1,100 kg ha^{-1} and 982 kg ha^{-1} more following faba bean compared to continuous wheat (Hailu et al., 1989; Asefa et al., 1992).

Amanuel and Tanner (1991) reported the importance of legumes in the cropping sequence to improve wheat yields and sustain production. Faba bean as precursor crop increased wheat grain yields, reduced the population of problematic grass weeds, and increased the income of farmers (Asefa et al., 2000). Improving crop productivity on a sustainable basis through crop rotation and improved management practices has been suggested as a sound management option for small-scale farmers. In the Horro highlands, farmers traditionally

0238–0161/$ 20.00

practice crop rotation based on short-term agronomic benefits from the precursor crops of wheat or the application of small amounts of fertilizers on low fertility soils (Asfaw et al., 1997). Asfaw et al. (1997) also reported that the traditional wheat-based cropping system is characterized by a one-year or two-year cropping sequence for wheat following cereals, legumes and oil seeds. Although the practice is well known, information on the type of precursor crops and their relative contribution has not been well studied for the different cultural management practices used in wheat production.

The use of legume precursor crops may be one of the possible options for increasing the productivity of wheat. Tanner et al. (1991) found that faba bean, field pea, rapeseed and linseed were the best precursor crops for wheat production. Legesse (2004) reported that the yield gap between experimental plots and demonstration fields was two-fold for wheat. The yield differences were attributed to variations in the management applied and in the use of inputs. Reddy (1996) reported that wheat productivity in Ethiopia is more constrained by poor crop management practices than by other production factors. It was further stated that the productivity of the best yielding varieties was sometimes equal to or even less than that improved local landraces. In the highlands of Horro, information on agronomic interventions for wheat production is scant. Hence, there is a need to consider cropping sequence and crop management factors that will enhance wheat productivity in the Horro highlands. Therefore, the objective of this study was to evaluate the effects of pulse and cereal precursor crops along with management levels on wheat production.

Materials and methods

A short-term crop rotation experiment was conducted during the1997–1999 cropping seasons at Shambo in the Horro Highlands. The area lies at 9°34'N latitude and 37°06'E longitude at an altitude of 2400 m.a.s.l. The mean annual rainfall is 1,695 mm (Fig. 1) (NMSA, 2003). It has a cool humid climate with mean minimum, mean maximum and average air temperatures of 8.15°C, 15.72°C and 11.94°C, respectively. The soil is a Nitisol.

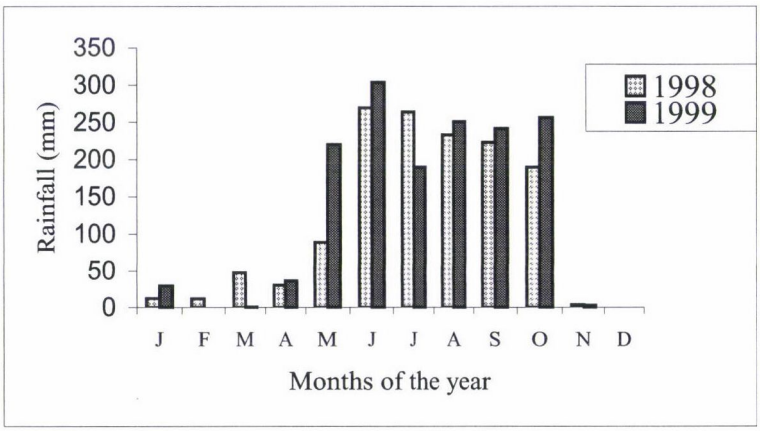

Fig. 1. Mean rainfall (mm) of the experimental site for two years

During the 1997 cropping season, the trial area was divided into two main plots and sown to precursor crops (barley and field pea). In the 1998 and 1999 cropping season each main plot from the 1997 field was divided into six equal parts and planted with two wheat varieties (local and improved). Each wheat variety was factorially combined with traditional and improved cultural management practices for wheat production.

The experiment was laid out as a split plot in a randomized complete block design with precursor crops as the main plots and management practices as the sub-plots. The precursor crops were field pea (*Pisum sativum)* variety G-22763-2c and barley (*Hordeum vulgare*) variety Shege. The management practices were: 1. farmer's variety and farmer's traditional practices without fertilizer (FVFP–FE); 2. improved variety and farmer's traditional practices without fertilizer (IVFP–FE); 3. farmer's variety with improved practices without chemical fertilizer (FVIP–FE); 4. improved variety with improved agronomic practices without chemical fertilizer (IVIP–FE); 5. farmer's variety with improved agronomic practices and chemical fertilizer (FVIP+FE); and 6. improved variety with improved agronomic practices and chemical fertilizer (IVIP+FE). The improved cultural practices were the practices recommended for wheat. The plot size was 10 m × 10 m. The wheat varieties used were the local variety *Molgo* (awnless wheat) and HAR-604 (Galama), an improved variety. The seed rate used was 160 kg ha^{-1} for the local variety (according to local farm practice) and 150 kg ha^{-1} for the improved variety. Sowing dates were between mid-June and early July. In the plots which received fertilizers, the recommended fertilizer rates of 100 kg ha^{-1} each of diammonium phosphate (DAP) and urea were applied at planting. For the improved cultural practices, the herbicide 2,4-D was applied 30 to 45 days after planting to control weeds. For the farmer's cultural practices, hand weeding was done once at 25 days after sowing. The data were analysed using MSTATC computer software. Mean separation was done using least significance difference (LSD) at the 5% probability level.

Selected orthogonal contrasts were used to separate the effects of management practices, fertilizer and variety. The six management practices were partitioned into the following orthogonal contrasts: farmer's variety vs. improved variety; with fertilizer vs. without fertilizer; and farmer's management vs. improved management levels. For economic evaluation, analyses were made of the partial budget, value to cost ratio (VCR), and marginal rate of return. Wheat grain was valued at EB* 137.00 100 kg^{-1}. The yield was down-adjusted by 10% to reflect actual production environments (CIMMYT, 1988). The wheat seed cost EB 2.40 kg^{-1} for the improved variety and EB 1.37 kg^{-1} for the local variety. Urea and DAP were valued at the official prices of EB 192 and 256 100 kg^{-1}, respectively. Other costs were labour for weeding at EB 3.50 day^{-1}, rent of the sprayer at EB 10.00 ha^{-1}, and herbicides at EB 0.42 $litre^{-1}$. *1€= 10.58EB.

Results and discussion

The combined grain yields across years averaged 1732 kg ha^{-1}, but the mean grain yields were higher in 1998 (1738 kg ha^{-1}) than in 1999 (1726 kg ha^{-1}). This may be due to the higher residual effect of the precursor crop in 1998 than in 1999 This reduction in the residual effect implies the need for fertilizer amendments to wheat during the second year of production.

The straw yield of wheat was significantly affected ($P<0.05$) by the precursor crop and by management practices but the interaction was non-significant (Tables 1 and 2). This confirms that the yield components of wheat are responsive to crop rotation and improved management practices. Amanuel et al. (2000) also reported that crop rotation had a significant effect on the yield components of wheat. The wheat straw yield was greater following field pea

than following barley precursor crop (Table 2). Amsal et al. (1997) reported that wheat following dicots had 54 % higher biological yield than wheat following cereals. The mean straw yield increased when fertilizer was applied, being greatest for FVFP+FE. The interaction effects on wheat straw yield were not significant, but the trend was for precursor crops to have a greater effect when fertilizer was applied.

Table 1
Mean squares of straw and grain yield of wheat due to precursor crops and management levels across two years at Shambo

Source of variation	DF	Mean squares	
		Straw yield (kg ha^{-1})	Grain yield (kg ha^{-1})
Year	1	20.267	2544.224
Precursor crop	1	1168.056**	4095522.058**
Year × precursor crop	1	2.569	163592.012*
Error	4	6.858	16097.833**
Management level	5	103.625**	928587.572**
Year × Management level	5	10.896	33853.555
Precursor crop × Management level	5	15.731	185437.999
Year × Precursor crop × Management level	5	4.043	27417.335
Error	40	11.541	175527.608

*,** Significant at 5% and 1% level of probability, respectively

Table 2
Effects of precursor crops and management levels on straw yield of wheat (kg ha^{-1}) at Shambo in 1998–1999

Treatment	Management levels						Mean
Precursor crop	FVFP–FE	IVFP–FE	FVIP–FE	IVIP–FE	FVFP+FE	IVIP+FE	
Barley	1217	1385	1713	1427	2290	1763	1632
Field pea	2142	2283	2260	2450	2743	2350	2438
Mean	1679	1834	2187	1938	2517	2057	
Yield change %	76	65	32	72	20	33	49

	Precursor crop	Management levels	Precursor crop vs. Management levels
$LSD_{(5\%)}$	121	280.3	NS
CV%	12.87	16.69	–

FVFP–FE= farmer's variety and farmer's traditional cultural practices without fertilizer, IVFP–FE = Improved variety and farmer's traditional cultural practices without fertilizer, FVIP–FE = farmer's variety with improved agronomic practices without fertilizer, IVIP–FE = Improved variety with improved agronomic practices without fertilizer, FVFP+FE = Farmer's variety with improved agronomic practices with fertilizer, IVIP+FE = Improved variety with improved agronomic practices with fertilizer, NS = non-significant

The main effect of precursor crops was significant for the grain yield of wheat (Tables 1, 3, 4 and 5). This indicates that precursor crops play a significant role in the grain yield of wheat. The wheat grain yield was higher following field pea than barley, by 39, 25 and 32% in 1998, 1999 and combined over the two years, respectively (Tables 3, 4 and 5). This result agrees with the findings of other authors (Hailu et al., 1991; Amanuel et al., 2000; Tilahun et al., 2000). Tanner et al. (1999) and Amsal et al. (1997) reported the wheat grain yield to be 22–54% and 59 % more following a dicot crop as compared to a cereal crop. The wheat yield after barley as precursor crop was not significantly different from continuous wheat production (Tanner et al., 1991). Amsal et al. (1997) found that wheat following any cereal (tef, wheat or barley) exhibited depressed yield and yield component performance and stunted plants. The better grain yield following field pea may be due to improved soil nitrogen availability (Martin et al., 1976; Alvey et al., 2001; Amanuel et al., 2000). The N requirement of wheat was higher following cereals than in sequences including leguminous crops (Singh and Singh, 1991). Tanner et al. (1991) reported that for most yield characters, the effect of faba bean as precursor crop was similar to that of N fertilizer. The higher wheat grain yield achieved following field pea demonstrated the significance of legumes for sustainable wheat production.

The management levels significantly ($P<0.05$) affected the grain yield (Tables 1, 3, 4 and 5). This indicated that improved crop management systems were required to maximise the wheat yield. The improved variety of wheat was observed to be more responsive to improved management levels in both fields. This is in agreement with the results of Amsal et al. (1999), who reported that improved cultivars are highly responsive to improved crop management systems. The improved variety gave greater grain yield under improved management levels both with and without fertilizers as compared to the local variety. The mean grain yield of the improved variety was greater than that of the local variety under both precursor crops. This confirms that the improved variety was more responsive to management practices and the cropping sequence. The increase in wheat grain yield due to management levels was greater after field pea than after barley. Martin et al. (1976) reported that crop rotation alone was unable to maintain yields at a high level unless the soil was fertile enough to maintain the potential productivity of the crop. The interaction between precursor crops and management practices was not significant ($P>0.05$) for the grain yield (Tables 1, 3, 4 and 5).

The mean wheat grain yield was higher following field pea than after barley for different management practices (Tables 3, 4 and 5), with grain yield advantages ranging from 24% (FVFP+FE) to 71% (IVIP–FE) and from 9% (FVFP+FE) in 1998 to 76% (IVIP–FE) in 1999 (Tables 3 and 4). The mean grain yield advantage for wheat following field pea as compared to barley ranged from16% for FVFP+FE to 73% for IVIP–FE (Table 5). This indicates that when the local variety was grown with traditional management practices and

fertilizers, it gave equivalent yields after both precursor crops. In contrast, with improved management but without fertilizers the improved variety gave a greater yield when grown after field pea than after barley. The use of field pea in sequential cropping system with improved management practices ensures the profitable, sustainable production of wheat.

Table 3
Effects of precursor crops and management levels on grain yield of wheat (kg ha^{-1}) at Shambo in 1998

Treatment	Management levels						Mean
Precursor crop	FVFP–FE	IVFP–FE	FVIP–FE	IVIP–FE	FVFP+FE	IVIP+FE	
Barley	1093	1563	1300	1400	1590	1763	1451
Field pea	1510	2250	1650	2390	1970	2373	2024
Mean	1302	1906	1475	1895	1780	2068	
Yield change %	38	44	27	71	24	35	39

	Precursor crop	Management levels	Precursor crop vs. Management levels
$LSD_{(5\%)}$	206	480	NS
CV%	8.25	22.94	–

Table 4
Effects of precursor crops and management levels on grain yield of wheat (kg ha^{-1}) at Shambo in 1999

Treatment	Management levels						Mean
Precursor crop	FVFP–FE	IVFP–FE	FVIP–FE	IVIP–FE	FVFP+FE	IVIP+FE	
Barley	1087	1800	1400	1210	1767	1947	1535
Field pea	1567	2033	1633	2133	1933	2200	1917
Mean	1327	1917	1517	1672	1850	2073	
Yield change %	44	13	17	76	9	13	25

	Precursor crop	Management levels	Precursor crop vs. Management levels
$LSD_{(5\%)}$	155	NS	NS
CV%	6.26		–

Table 5
Effects of precursor crops and management levels on mean grain yield of wheat (kg ha^{-1}) at Shambo (combined over two years, 1998–1999

Treatment	Management levels						Mean
Precursor crop	FVFP–FE	IVFP–FE	FVIP–FE	IVIP–FE	FVFP+FE	IVIP+FE	
Barley	1090	1681	1350	1305	1678	1855	1493
Field pea	1538	2142	1642	2262	1952	2287	1970
Mean	1314	1911	1496	1783	1815	2071	
Yield change %	41	27	22	73	16	24	32

	Precursor crop	Management levels	Precursor crop vs. Management levels
$LSD_{(5\%)}$	83.02	345.7	NS
CV%	7.33	24.19	

NS = non-significant

Orthogonal contrasts between local and improved varieties of wheat showed a highly significant mean grain yield difference ($P<0.05$ and 0.01) (Table 6), the improved variety resulting in a mean grain yield increase of 190 kg ha^{-1}. This indicated that the productivity of the improved variety of wheat was more sensitive to management levels than the local variety. The contrast made between the variant with fertilizer and that without fertilizer indicated a highly significant effect ($P<0.01$) on the mean grain yield of wheat and resulted in a yield advantage of 106 kg ha^{-1} from applied fertilizer. Improved agronomic management vs. farmers' management level revealed a highly significant effect ($P<0.01$) on the mean grain yield of wheat and resulted in a yield advantage of 60 kg ha^{-1} from improved management practices. Thus, improved agronomic practices are advantageous for wheat production in the Horro highlands.

The result of economic analysis for precursor crops indicated that the highest net benefit was from wheat following field pea (EB 2551 ha^{-1}) compared to barley (EB 1923 ha^{-1}) (Table 7). Wheat following field pea thus gave an advantage of EB 628 ha^{-1}, or 33% as compared to barley as precursor crop (Table 7). Thus, field pea as precursor crop gave a high net benefit for wheat production as compared to barley.

Economic analysis for management practices indicated that the highest net benefit (EB 1466 ha^{-1}) and value to cost ratio (EB 2.94 profit per unit of investment) were obtained from FVIP–FE (Table 8). The second highest net benefit (EB 1433 ha^{-1}), with a value to cost ratio of EB 1.16 profit per unit investment, was achieved with IVIP+FE (Table 8). The value to cost ratio ranged from 1.01 to 2.94 for management practices (Table 8). In the current study the low cost of grain resulted in the farmer's variety dominating at the given cost level. The high cost of commercial fertilizer and improved seed made the improved agronomic practices for wheat production costly. Both the local and improved varieties gave better grain yield under intensive management practices with fertilizer application (Table 5). These findings indicate that improved management practices boost the yield of wheat.

Table 6

Effect of management levels, fertilizer and variety on wheat mean grain yield at Shambo, 1998–1999

Contrast[a]	Mean grain yield
Farmer's vs. improved management level	**
Local vs. improved variety	**
With fertilizer vs. without fertilizer application	**

** Significant at 1% level of probability; [a] See materials and methods for complete description of orthogonal contrasts used

Table 7
Partial budget and net benefit analyses for precursor crops on the mean grain yield of wheat at Shambo

Items	Precursor crop	
	Barley	Field pea
Average wheat yield (kg ha^{-1})	1493	1970
Adjusted wheat yield (kg ha^{-1})	1343.7	1773
Gross field benefit of wheat (EB ha^{-1})	1841	2429
Average straw yield (kg ha^{-1})	1632	2438
Gross field benefit of wheat straw	81.6	121.9
Total field benefit (EB ha^{-1})	1923	2551
Net benefit	1923	2551

Note: Grain price = EB 1.37 kg^{-1}, Seed price = EB 2.40 kg^{-1} for improved variety, Seed price = EB 1.37 kg^{-1} for local variety, Straw cost = EB 0.05 kg^{-1}, Yield was down–adjusted with a coefficient of 10%, EB = Ethiopian Birr, 1€ = 10.58EB

Table 8
Partial budget and marginal rate of return (MRR) analyses for the effects of management levels on the grain yield of Wheat at Shambo, 1998–1999

Item	Management levels					
	FVIP–FE	FVFP–FE	FVIP+FE	IVIP–FE	IVFP–FE	IVIP+FE
Average yield (kg ha^{-1}) of wheat	1496	1314	1815	1783	1911	2071
Adjusted yield (kg ha^{-1}) wheat	1346.4	1182.6	1633.5	1604.5	1719.9	1863.9
Gross field benefit of wheat grain	1845	1620	2238	2198	2356	2554
Average straw yield (kg ha^{-1})	2187	1679	2517	1938	1834	2057
Gross field benefit of wheat straw	120.00	92	138	107	101	113
Total field benefit (EB ha^{-1})	1965	1712	2376	2305	2457	2667
Wheat seed cost (EB ha^{-1})	205.50	205.50	205.50	360.00	360.00	360.00
Urea	–	–	192.00	–	–	192.00
DAP	–	–	256.00	–	–	256.00
Rental price of sprayer (EB ha^{-1})	10	–	10	10	–	10
Herbicide cost (EB ha^{-1})	42	–	42	42	–	42
Fertilizer application cost (EB ha^{-1})	–	–	10.5	–	–	10.5
Total labour cost (EB ha^{-1})	241	645	350	682.5	756.5	364
Total Costs that vary (EB ha^{-1})	498.50	850.50	1066.00	1094.5	1116.5	1234.00
Net benefit	1466.50	861.5^{D}	1310^{D}	1210.5^{D}	1340.5^{D}	1433^{D}
Value to cost ratio	2.94	1.01	1.23	1.11	1.20	1.16

Note: D = dominated treatment, Urea = EB 1.92 kg^{-1}, DAP = EB 2.56kg^{-1}, Labour cost = EB 3.5 day^{-1}, 2,4-D = EB 0.42 litre^{-1}, Grain price = EB 1.37 kg^{-1}, Local seed = EB 1.37 kg^{-1}, Improved seed = EB 2.40 kg^{-1}, Straw cost = EB 0.05 kg^{-1}, Rental price of sprayer = EB 10.00 ha^{-1}, Fertilizer application cost = EB 10.50 ha^{-1}, Yield was down-adjusted with a coefficient of 10%, EB = Ethiopian Birr, 1€ = 10.58EB

Conclusions

Much higher wheat yields were obtained following field pea as precursor crop than after barley. The grain yield was increased by fertilizer use and sowing the improved variety, but the yield increases were not sufficient to improve

profitability over sowing the local cultivar with no fertilizer applied. The most important factor in profitability is to adopt the recommended cultural practices. The economic analysis indicated that optimum market prices should be determined by policy-makers for wheat producers in order to exploit the potential benefit of package recommendations. Alternative technologies should be developed to integrate the use of inorganic and organic fertilizers for profitable wheat production. Therefore, long-term investigations on the economic benefit of the rotation effect with intensive management levels and extending the recommendations to users is essential for sustainable wheat production in the Horro highlands.

Acknowledgements

The authors are grateful to the Agricultural Development Bureau of Oromiya National Regional State for funding the project, to A. Gemeda and A. Mussa for their valuable advice, to D. G. Tanner, T. Asefa, G. Diriba, D. Temesgen and A. Girma for their comments on the manuscript, to A. Mijena, T. Tesso and Y. Kenea for their technical assistance, and to Bako Research Center Management for facilities for the field trial.

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Acta Agronomica Hungarica, 53(3), pp. 283–291 (2005)

YIELD FORMATION IN COMMON BUCKWHEAT (*FAGOPYRUM ESCULENTUM* MOENCH)

J. KALINOVÁ, J. MOUDRÝ and V. ČURN

FACULTY OF AGRICULTURE, UNIVERSITY OF SOUTH BOHEMIA, ČESKÉ BUDĚJOVICE, CZECH REPUBLIC

Received: 15 February, 2005; accepted: 21 September, 2005

Seven varieties of common buckwheat were tested in field experiments under two levels of nitrogen fertilisation (0 and 50 kg N ha^{-1}) during the period 1998–2000. The objective of the experiments was to evaluate the influence of varieties, fertilisation and year on the formation of yield components in common buckwheat and to evaluate the relationships between the individual components. As was observed, a compensatory ability exists among the particular yield components. An increase in stand density reduces branching and the number of inflorescences per plant. The number of flowers in the raceme is influenced by the mean day temperature during the period from flower bud formation to the beginning of flowering ($r = -0.98$, $P < 0.01$). Rainfall during flowering negatively influenced the number of achenes per plant. The high number of flowers reduced seed setting and negatively influenced the yield due to competition for assimilates. The high number of achenes per plant was linked to lower weight ($r = -0.33$, $P < 0.05$). The occurrence of empty achenes was a result of the susceptibility of the plants to the temperature conditions during the achene maturation period ($r = 0.86$, $P < 0.05$). The main yield component determining the yield was the number of fully developed achenes per plant.

Key words: *Fagopyrum esculentum,* yield components, varieties, nitrogen fertilisation

Introduction

Common buckwheat (*Fagopyrum esculentum* L.) was introduced in the territory of the current Czech Republic in the 12th century. Interest in growing it has revived in recent years due to its nutritional composition and typical low-input crop characteristics.

Buckwheat has high potential productivity, as one plant forms about 400 to 1000 flowers, but in practice it exhibits very low productivity (Jafimenko and Barabaš, 1990). Although the world mean yield doubled over the last 40 years and reached 1.2 t ha^{-1} in 2000 in comparison to 0.5 t ha^{-1} in 1961 (according to FAO), the yield level is very low as compared with common cereals. Buckwheat exhibits great differences in the yield of individual plants and these differences cause a high variation in the total yield of achenes. Some plants may form up to 200 seeds and others only about ten (Edwardson, 1996). The low number of fully developed seeds per plant is caused by the high proportion of flowers that dry up. This is mainly due to self-incompatibility, heterostyly, insufficient pollination, poor distribution of assimilates, branching and undetermined

growth, causing strong competition for nutrients between developing seeds and flower buds (Adhikari, 1997). Further losses are caused by defects during the embryonic development of the achenes (Guan and Adachi, 1992). The long period of flowering (4–6 weeks) exposes buckwheat to changing weather conditions. The productivity of buckwheat also depends on the traits of different varieties and on their response to ecological conditions (Alekseeva, 1986). Low yield is also explained by a shortage of varieties adapted to particular climatic and soil conditions (Jafimenko and Barabaš, 1990).

The main yield components in common buckwheat are the number of inflorescences per square metre, the number of achenes per inflorescence and the thousand-achene weight.

Buckwheat is able to utilise nutrients non-available for other crops. It takes 34, 16 and 40 kg of N, P_2O_5 and K_2O, respectively, from the soil per ton of achene yield. Thus, a nitrogen dose of 20–50 kg ha^{-1} was recommended, depending on the preceding crop and soil fertility (Petr and Hradecká, 1997).

The aim of the present work was to evaluate the influence of varieties, N fertilisation and year on the formation of yield components in common buckwheat and to evaluate relationships between the individual components.

Materials and methods

Seven varieties of common buckwheat (Pyra – CZ, 2n; Aelita – RUS, 2n; Emka – PL, 4n; Huszowska – PL, 2n; Kora – PL, 2n; Krupinka – RUS, 2n; Sumčanka – RUS, 2n) were tested in field experiments in České Budějovice (380 m above sea level, acid cambisol, gleying sandy loam soil) under two levels of nitrogen fertilisation (0 and 50 kg N ha^{-1} as ammonium nitrate with limestone) during the period 1998–2000. Soil pH was in the range of acid to moderately acid (pH 5.0–6.6), and the mean content of soil mineral nitrogen (NH_4^+, NO_3^-) was low to medium (8–23 mg kg^{-1}). Basic soil characteristics and basic meteorological data are given in Tables 1 and 2.

Buckwheat was sown in the middle of May in 12.5 cm wide rows, with 200 plants per m^2 density. The area of each plot was 10 m^2, and each variant had four replicates. No mechanical or chemical treatment was applied during the vegetation period.

Thirty plants were sampled before harvest from each variant and replicate. From these 30 plants, ten mean plants were analysed and the number of primary branches, and the number of full and empty achenes were counted. The number of flowers per raceme was recorded during the full flowering of the plants. The thousand-achene weight was determined after harvest in all four replicates.

The data were analysed using analysis of variance, Tukey's HSD test and correlation analysis (Statistica 6.0 software).

Table 1
Basic soil characteristics, pH values and mineral content (mg kg^{-1})

Year	pH/KCl	P	K	Mg	Ca	Na	Zn	Cu	Fe	N-NH_4	N-NO_3
1998	6.3	72	107	154	1330	328	40	17.3	16758	3.6	12.1
1999	6.3	91	125	144	1364	290	172	29.4	854	2.4	10.5
2000	5.5	144	128	96	1033	50.6	108	32.7	1305	3.9	11.2

Table 2
Basic meteorological data during the period 1998–2000

	April	May	June	July	August	September	Mean*	Year mean
Mean temperature (°C)								
1998	9.8	14.3	17.8	18.3	18.5	12.9	16.1	9.3
1999	9.3	14.6	16.3	19.5	17.9	16.3	14.6	9.3
2000	12.0	15.6	18.6	16.5	19.3	14.0	15.9	9.9
30-year mean	8.1	13.0	16.2	17.7	17.1	13.5	14.3	8.2
Precipitation (mm)								
1998	40.2	37.9	109.9	93.0	35.4	64.5	341	579.5
1999	22.9	75.1	43.5	72.9	68.2	41.1	336.2	505.1
2000	6.7	45.2	68.6	104	73.2	72.0	369.6	601
30-year mean	46.5	70.1	93.0	77.8	78.8	47.5	413.7	528.8

*for the vegetation period

Results

A lower number of plants per m^2 (Table 3) and greater variability in the stands were observed in 1999 due to the dry period during buckwheat emergence (Table 2). The influence of nitrogen fertilisation on the plant number per m^2 was not statistically significant ($P > 0.05$) (Table 4).

Neither the varieties nor the nitrogen fertilisation treatments caused any difference in the number of branches, number of inflorescences, number of flowers, number of fully developed and empty achenes per plant or in the yield (Table 4).

Table 3
Yield and yield components of common buckwheat

	1	2	3	4	5	6	7	8
Variety								
Aelita	124	1.37	11.52	69.70	44.72	35.59	26.17	0.99
Emka	135	1.69	12.05	61.84	37.53	39.55	32.47	0.89
Hruszowska	128	1.50	12.89	75.68	45.64	40.52	25.49	0.98
Kora	124	1.31	13.01	57.78	42.42	41.68	26.28	1.07
Krupinka	132	1.03	11.58	65.73	37.82	45.79	25.81	1.05
Pyra	124	1.16	13.72	63.53	40.94	38.77	25.57	0.96
Sumčanka	116	1.36	13.26	67.42	37.10	32.57	25.44	0.85
N fertiliser								
0 kg ha^{-1}	128	1.29	12.25	66.04	38.08	34.62	26.66	0.98
50 kg ha^{-1}	125	1.40	12.90	65.87	43.68	43.80	26.84	0.96
Year								
1998	136	0.92	9.24	74.66	22.41	29.85	27.87	0.42
1999	99	2.19	18.04	67.48	57.23	62.72	25.69	1.05
2000	145	0.92	10.45	55.74	43.02	25.06	26.68	1.45

1: No. of plants per m^2; 2: No. of primary branches per plant; 3: No. of inflorescences per plant; 4: No. of flowers per inflorescence; 5: No. of fully developed achenes per plant; 6: No. of empty achenes per plant; 7: Thousand-achene weight (g); 8: Yield (t ha^{-1})

The influence of year on the yield and yield components was statistically significant ($P < 0.01$) (Table 4).

The highest mean number of branches and inflorescences per plant (1.3 and 12.7, respectively) was observed in 1999 (Table 3). The differences between years were also affected by the stand density. There was a low number of plants per square metre in 1999, allowing the formation of a higher number of branches per plant. A significant negative correlation ($P < 0.01$) was recorded between the numbers of plants and branches and a positive correlation ($P < 0.01$) between the numbers of branches and inflorescences (Table 5).

The number of flowers per plant ranged from 600 to 1200. The highest number of flowers per inflorescence (67.5) was recorded in 1998. A period with plentiful precipitation and a mean temperature of 15.7°C preceded buckwheat flowering in that year.

A wide range of 4–134 fully developed achenes per plant was recorded. The highest mean number of achenes per plant (57.2) was observed in 1999 (Table 3). The achene number per plant was influenced by the lower stand density in that year. After rainfall during the period of buckwheat flowering in early June 1999, high temperatures followed during the period of achene formation. Thus, the achenes originated from inflorescences formed during the later stages of the vegetation period. The highest number (62.7) of empty achenes was recorded in 1999 (Table 3). Statistically significant positive correlations ($P < 0.01$) were found between the number of branches and inflorescences per plant and between the number of branches and achenes per plant (Table 5). On the other hand, a negative correlation ($P < 0.01$) was found between the number of plants per square metre and the number of full achenes per plant.

Table 4
Analysis of variance (MS effect)

Factor	df	1	2	3	4	5	6	7	8
Variety	6	431.20**	386.70	200.55	271.5	15.72	400.37	1.13	0.15
Fertilisation	1	0.12	124.55	0.30	1181.0	15.43	2077.2	0.54	0.04
Year	2	65.39**	20083**	1277.5**	17041**	1238**	30601**	29.6**	15.1**
Variety × fertilisation	6	1.84	200.94	92.18	406.2	9.02	248.95	0.13	0.15
Variety × year	12	21.27**	104.17	408.90	472.3	28.67	347.70	0.85	0.30**
Fertilisation × year	2	4.14*	131.15	1.81	714.0	30.97	161.92	0.01	0.26
Variety × fertilisation × year	12	3.36*	292.69	362.15	465.2	18.96	335.97	0.68	0.13

1: Thousand-achene weight (g); 2: No. of plants per m^2; 3: No. of flowers per inflorescence; 4: No. of fully developed achenes; 5: No. of inflorescences; 6: No. of empty achenes; 7: No. of primary branches; 8: Yield (t ha^{-1}); ** $P < 0.01$, * $P < 0.05$

Table 5
Correlations between yield components of common buckwheat

Yield components	Yield	1	2	3	4	5	6
Number of plants per m2	0.05						
Number of inflorescences	0.19	–0.71**					
No. of flowers per inflorescence	–0.33*	–0.13	–0.01				
Number of branches	0.07	–0.76**	0.72**	0.12			
No. of empty achenes	0.06	–0.56**	0.68**	0.01	0.61**		
No. of fully developed achenes	0.52**	–0.57**	0.53*	–0.21	0.58**	0.50**	
Thousand achene weight	–0.29	0.30	–0.20	–0.11	–0.08	–0.18	–0.33*

1: No. of plants; 2: No. of inflorescences; 3: No. of flowers per infloresence; 4: No. of branches; 5: No. of empty achenes; 6: No. of fully developed achenes; ** $P < 0.01$, * $P < 0.05$

The lowest value of thousand-achene weight (TAW) was observed in 1999 (Table 3), when the highest number of achenes per plant was also registered. The highest mean TAW value was determined in 1998. A negative correlation ($P < 0.05$) was found between achene weight and the number of achenes per plant (Table 5). Significant differences ($p < 0.01$) were found between the tested varieties. A considerably higher weight was recorded in the tetraploid variety Emka.

The highest total yield of achenes (1.45 t/ha) was observed in 2000 (Table 3). In that year, by contrast to the other years, there was no rainfall during the flowering period. Buckwheat plants had higher productivity when there was a lower number of flowers and branches and medium levels of TAW, achene number and inflorescence number. There was a negative correlation ($P < 0.05$) between the number of flowers per inflorescence and the yield, and a positive correlation ($P < 0.01$) between the number of full achenes and the yield (Table 5).

Discussion

A lower number of plants per square metre was recorded in the field experiments as a consequence of the long dry period, which was unfavourable for buckwheat emergence. The number of plants per square metre is affected by the sowing rate, emergence conditions and biological quality of the seeds (Petr and Hradecká, 1997). Ruszkowski (1990) concluded that buckwheat needed soil moisture mainly during the initial 20 days after sowing, with optimum precipitation being 70 mm.

Higher branching was reported when a large proportion of the nutrients was utilised for vegetative growth and only a small part for the accumulation of assimilates (Kreft, 1989). The highest number of branches per plant was observed in the year when there was also the lowest number of plants per square metre. Increasing plant density was found to reduce the branching of the individual plants. The most productive plant ideotype has only a main stem

without primary or secondary branches (Ruszkowski, 1990). As confirmed in the present experiments, the plants reached higher productivity with a lower number of branches under favourable conditions during flowering, when rainfall did not inhibit pollination and thus did not negatively influence the formation of achenes. Optimum density appears to be an important factor influencing the yield level. The lower number of branches in 1998 as compared with 2000 can be explained as a consequence of the limited rainfall during the branching period. The formation of branches of the first order begins after the appearance of the second leaf. The stems form branches until the creation of the first flower node forming inflorescences (Jakimenko, 1982).

Nitrogen fertilisation did not prove to be an efficient factor in the present experiments. Noworolnik (1995) concluded that the efficacy of applied nitrogen depends on the soil type, nutrient supply and level of precipitation. Nitrogen application was found to be most profitable under moderate rainfall and poor soil conditions. The nitrogen content in the soil of the present experiment was medium and the rainfall levels were similar to or below the long-term mean. Another important factor influencing the number of inflorescences was the year. Hradecká (1995) reported that a sufficient amount of rainfall during the period of inflorescence formation (from the first pair of leaves to the start of flowering) resulted in a higher number of racemes per plant. In the present experiments no significant correlation was observed between precipitation and the number of inflorescences ($P > 0.05$). The highest number of inflorescences was observed in 1999, regardless of the low precipitation from sprouting to flowering (Table 3). Adhikari (1997) reported a positive correlation between the number of branches and number of inflorescences, which was confirmed in the present work. A statistically significant correlation ($P < 0.01$) was also found between the number of inflorescences and the number of plants per m^2. The transmission of light into the buckwheat stand is poorer at higher stand density and the number of inflorescences is reduced (Ruszkowski, 1990). A higher number of achenes developed in the larger number of inflorescences, but their weight was lower.

A highly significant negative correlation ($r = -0.98$, $P < 0.01$) was recorded between the number of flowers per raceme and the mean day temperature in the period between flower bud formation and the beginning of flowering. These results are similar to those of Hradecká (1995). The correlation between precipitation from emergence to flower bud formation and the number of flowers per inflorescence was not statistically significant, thus contradicting the findings of Hradecká (1995) that optimum precipitation before flower bud formation increased the number of flowers per raceme.

Ruszkowski (1990) found the highest number of full achenes in inflorescences formed at a later stage. In the present experiments, too, the highest number of achenes per plant was recorded when the rainfall during flowering was followed by higher temperatures, resulting in the forcing of later inflorescences, which were formed mainly on branches. The optimum

temperature for pollination is 20°C (Sugarawa, 1956). The number of achenes per plant was influenced by the stand density, plants forming fewer branches and inflorescences in dense stands. Ruszkowski (1990) reported that the number of seeds per plant was lower at higher stand density. A dense stand also prevents insect pollinators from gaining access to the flowers. Branching and the formation of later inflorescences due to high stand density did not compensate for the losses. Kusiorska and Koszykowska (1981) reported the drying up of pollinated flowers and the consequent low seed setting as the limiting factors influencing yield. In the present work a highly significant negative correlation ($r = -0.91$, $P < 0.01$) was found between the sum of precipitation during flowering and achene setting, and a significant positive correlation ($r = 0.83$, $P < 0.05$) between the mean day temperature during flowering and achene setting. This meant that under the given experimental conditions the temperature was not the main factor influencing the drying up of flowers, as reported by Petr and Hradecká (1997). This negative characteristic was influenced by the competition for assimilates between the vegetative and generative parts of the plant.

A certain proportion of the achenes does not fully develop, leading to a number of empty achenes. Ruszkowski (1990) attributed achene abortion to the weakening of the self-incompatibility mechanism during the last stages of flowering. The number of empty achenes increased with an increase in the number of inflorescences and branches per plant and decreased with increasing stand density. The formation of a large number of achenes leads to competition for assimilates, forcing some achenes to abort their development. The formation of empty achenes can be explained by the selection process and by susceptibility to unfavourable climatic conditions such as high temperature and/or insufficient precipitation (Guan and Adachi, 1992). In the present experiments, the highest number of empty achenes was observed when the mean temperature during the achene setting period reached 20°C. This temperature seems to be optimal for pollination but too high for achene development. During maturing the optimum temperature is 17–20°C with short periods of rainfall (Ruszkowski, 1990). A statistically significant correlation ($r = 0.86$, $P < 0.05$) was found between mean temperature during achene development and the number of empty achenes. However, the opinion that tetraploid varieties produce a higher number of empty achenes than diploid ones was not confirmed (Guan and Adachi, 1992).

The TAW value was influenced by the number of achenes per plant. The lowest value was observed in the year when there was the highest number of achenes per plant. By contrast, the highest TAW value was determined in the year in which the lowest number of achenes per plant was recorded. Low achene weight can be explained by the competition for nutrients. The TAW value is influenced by temperature, precipitation, health status and the availability of nutrients during the achene formation period (Jafimenko and Barabaš, 1990). Colder, wet climatic conditions allow the better development of achenes with higher weight (Hradecká, 1995). The highest achene weight was recorded in the tetraploid variety Emka.

The highest total yield of achenes was associated with the lowest number of flowers per plant. There was dry sunny weather during flowering. The decreased number of flowers was caused by extremely high "tropical temperatures" in the first ten days of June during flower formation. If the number of flowers is too high it causes strong competition for assimilates and a decrease in seed formation, thus having a negative influence on the yield. In the present experiments, the main yield component in buckwheat was the number of fully developed achenes. Thus, the weather conditions during the periods of flower formation and flowering can be specified as the crucial factor affecting yield.

As in the present experiments, no substantial yield differences were observed as the result of different doses of nitrogen fertilisation by Zakarackas (1999) or Aufhammer et al. (1994). Thus, buckwheat does not use the applied nitrogen for achene formation, probably due to its rapid, incomplete development.

No yield differences were observed between the tested buckwheat varieties. This was probably due to cross-pollination, great variability within each variety and good ability to respond to environmental conditions. The climatic conditions are considered to be the main factor influencing common buckwheat development. Differences between varieties were reported by Aufhammer and Kübler (1991), who compared varieties with complete and incomplete development (European and Asian cultivars).

Acknowledgements

This work was supported by grants from the Ministry of Education of the Czech Republic (MSM: 12220002 and 12220004).

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Acta Agronomica Hungarica, 53(3), pp. 293–301 (2005)

ALUMINIUM–PHOSPHORUS INTERACTIONS ON GROWTH AND SOME PHYSIOLOGICAL TRAITS OF CARROT AND RADISH PLANTS

M. ISMAIL

BOTANY DEPARTMENT, FACULTY OF SCIENCE, SOUTH VALLEY UNIVERSITY, QENA, EGYPT

Received: 24 August, 2004; accepted: 19 August, 2005

Aluminium treatment caused a significant decrease in root length and dry matter yield in the shoots and roots of carrot (*Daucus carota* L.) and radish (*Raphanus sativus* L.) plants. This reduction was concomitant with a decrease in the accumulation of soluble sugars and total amino acids, whereas a significant increase in the proline content of the shoots and roots was detected. Soluble protein remained more or less unchanged when Al was applied at low and moderate levels. However, at higher Al levels, the losses in soluble sugars were accompanied by increases in soluble protein in radish, whereas in carrot the opposite effect was observed.

The application of phosphorus fertilizer to Al-treated plants counteracted the toxic effect of aluminium by increasing root elongation and dry matter production. This was associated with high contents of soluble sugars and soluble protein as well as Ca, Mg and P, especially at low and moderate levels of aluminium.

Key words: aluminium toxicity, carrot (*Daucus carota* L.), dry matter, radish (*Raphanus sativus* L.), root elongation

Introduction

Aluminium toxicity is a growth-limiting factor for plants grown on acid soils (Foy, 1992). Acid soils are often characterized by high concentrations of aluminium, low total and available phosphate content, and high P retention capacity. The inhibition of root elongation is the primary initial response to Al toxicity (Kochian, 1995; Matsumoto, 2000). The reduction of the root system makes crops more sensitive to other abiotic stresses such as water and nutrient stress and ultimately reduces crop yield (Granados et al., 1993). The symptoms of Al rhizotoxicity include stunted roots, poor root hair development and swollen root apices (Tang et al., 2001). Higher proline content has been associated with plants grown with excess Al compared to no Al (Zaifnejad et al., 1997). Proline increases were greater in Al-sensitive than in Al-tolerant genotypes (Klimashevskii, 1983). Basu et al. (1994; 1997) reported a decrease in amino acid yield in response to Al stress in wheat plants. In addition, Silva et al. (2001) suggested that Al nutrient solution had an inhibitory effect on the uptake of Ca and Mg and their translocation (or allocation) into various organs of the plants

On the other hand, the application of phosphorus as a fertilizer to soils could decrease Al toxicity, due to the direct precipitation of aluminium phosphates in the zone of P incorporation. In addition, it has been shown that

0238–0161/$ 20.00

soluble, yet non-toxic Al-P complexes may form in solution (Alva, 1986). Generally, the chemical interactions of Al with phosphate are able to lower the activity of Al^{3+} in the soil solution (Sanzonowicz et al., 1998; Silva et al. 2001; Nakagawa et al., 2003). However, in most soils, P is concentrated at the soil surface, while high concentrations of phytotoxic Al are found only at depth. Also, surface applied P has been found to improve root penetration into acidic subsoils where a high concentration of aluminium would otherwise limit root extension (de Miranda and Rowell, 1987; Gaume et al., 2001). McLaughlin and James (1991) also postulated that P nutrition may affect resistance to Al through changes in the cation-anion balance within plants.

The objective of this research was to assess the effects of Al and P addition on the growth, mineral contents and some metabolic mechanisms of carrot and radish plants.

Material and methods

The effect of aluminium stress on the seedling growth of carrot (*Daucus carota* L.) and radish (*Raphanus sativus* L.) plants was studied. The following aluminium stresses were used: 0, 200, 400, 600 and 800 mol/L Al as $AlK(SO_4)_2$ in 0.1 N Hoagland solution. Seeds of the control group were germinated using only 0.1 N Hoagland solution as a substrate. To evaluate the interactive effects of P as KH_2PO_4 with Al, 10 mM KH_2PO_4 was added with the previous Al treatments.

Twenty seeds of carrot and radish were sterilized with absolute ethyl alcohol for 1 min, washed in 10% clorox (5.25% sodium hypochlorite) for 5 min and then rinsed several times in deionized water. Thereafter they were germinated in Petri dishes at about 25°C. Three replicate Petri dishes were prepared for each treatment.

Twenty seeds were placed between folded paper towels, covered by plastic film, rolled up, and placed upright in 600 mL beakers. Eighty mL of solution were used to saturate the towels in each of the treatments, and the seeds were treated in the same manner as described above. The plants were allowed to grow in a growth chamber at 28/22°C day/night (12 h) temperature cycles with a light intensity of 105 mol m^{-2} s^{-1} for three weeks. Distilled water was added as needed to compensate for evaporation loss. The plants were harvested after three weeks. Ten plants from each treatment were divided into roots and shoots. Their lengths and weights were recorded, after which they were dried in an oven at 80°C to constant weight.

Total soluble sugars were estimated using the anthrone method (Plummer, 1978). For extraction, 0.1 g frozen sample was randomly taken and added to 9 ml HCl (2N). The tubes were placed in a boiling water bath for 3 h with a marble on top to prevent loss of water by evaporation. After cooling, the sample was transferred to a calibration flask (50 ml) and 3 ml $ZnSO_4$ (10%) was added to each flask. The sample was treated with 5 ml KOH (2N) and neutralized with H_2SO_4 (0.05N). The volume of the sample was then made up to 50 ml with distilled water. The sample was filtered, then 1 ml of the filtrated sample was added to 4 ml anthrone reagent (2 g/L in conc. H_2SO_4). The tubes were placed in a boiling water bath for 10 min, then cooled. The extinction was read at 620 nm against a reagent blank, using a spectrophotometer (*Spectronic Genesys ZPC*, Rochester, NY, USA). Concentrations of soluble sugar were calculated with reference to a calibration curve established with a standard solution of glucose (0.1 g/L).

Soluble protein was determined according to Lowry et al. (1951). Free amino acids were determined according to the method of Moore and Stein (1948). The proline content was estimated according to the method described by Bates et al. (1973). Dried plant parts (50 mg) were ignited at 500°C in an oven for 4 h. The ash was dissolved twice with HNO_3 (1:3 v/v) and finally dissolved

in HCl (1:3 v/v) with distilled water to achieve a final HCl dilution of 1:30. Calcium was analysed by flame photometry (*Jenway PFP 7*, Essex, UK) and Al, Mg and P by atomic absorption (Spectra 55, Variane Type).

The experiments were carried out in a completely randomized design with three replications (10 plants each). The experimental data were analysed using the least significant difference test.

Results

The data presented in Table 1 reveal that the lengths of roots and shoots, as well as the dry matter yield of the carrot seedlings remained unaffected up to 200 mol/L Al, but above this level the values tended to decrease as compared with the control plants. Radish seedlings were more severely affected by aluminium toxicity, and growth was completely inhibited at 800 mol/L Al. However, the shoot/root ratio (S/R) increased with increasing Al concentration. On the other hand, the application of phosphate (10 mM KH_2PO_4) to Al-treated plants caused a significant increase in all these parameters compared with the corresponding aluminium treatments. Moreover, P application not only alleviated the toxicity effect of Al stress on the growth parameters, but at some levels overcompensated. This stimulatory effect was more obvious in carrot than in radish plants.

The content of soluble sugars in the shoots and roots of carrot plants remained more or less unaffected up to a level of 400 mol/L Al, after which it significantly decreased with increasing aluminium treatments (Table 2). In the case of radish plants, all the aluminium levels resulted in progressively lower contents of soluble sugars than found in the control samples (Table 3). The phosphorus-aluminium interaction markedly increased these values in both test plants when compared with the corresponding treatments with Al only. Also, the data in Table 2 reveal that the soluble protein content of Al-treated carrot plants remained more or less unchanged up to 200 mol/L Al and then progressively decreased with increasing Al levels. In the case of radish plants the soluble protein content was markedly higher than that of the control, irrespective of the level of Al treatment (Table 3). The proline content of the two test plants (Tables 2 and 3) showed progressively greater accumulation, above that of the control, with increasing aluminium concentration in the culture medium. The other amino acids of the two test plants tended to decrease with the rise in aluminium level and this reduction was more obvious at moderate and higher aluminium levels. An important observation was that radish plants accumulated more proline and other amino acids than carrot plants. In addition, this accumulation was more pronounced in the roots than in the shoots in the two experimental plants. However, the application of P fertilizer significantly reduced the proline accumulation at each of the aluminium levels in both test plants, whereas an opposite pattern was exhibited by the contents of other amino acids (Tables 2 and 3).

Table 1

Effect of Al stress and P fertilization on shoot and root lengths (cm seedling^{-1}), dry matter (g seedling^{-1}) and shoot dry matter/root dry matter in shoots and roots of carrot and radish plants

Treatments	Carrot					Radish				
	SL	RL	SDM	RDM	SDM/RDM	SL	RL	SDM	RDM	SDM/RDM
Al (mol/L)										
Control	15.25	11.20			1.13	12.35	9.44	11.72	9.07	1.29
200	14.70	10.75	13.16	11.44	1.15	10.45*	7.15**	9.88*	6.63**	1.49**
400	13.30*	10.15	12.67	10.96	1.16	8.55**	5.50**	7.83**	5.12**	1.53**
600	12.75**	5.80**	8.64**	7.11**	1.22*	7.25**	4.60**	4.91**	2.65**	1.85**
800	10.50**	4.20**	7.83**	5.14**	1.52**	IE	IE	IE	IE	IE
10 mM KH_2PO_4										
Control	17.40*	13.10*	16.47*	14.10*	1.17	13.80	10.55	12.34	10.38	1.19**
200	15.75	11.40	15.76	12.63	1.25**	12.22	9.80	11.97	9.71	1.23*
400	15.40	11.45	14.48	10.32*	1.40**	10.85*	7.90*	9.74*	7.26*	1.34*
600	13.15**	8.75**	11.33*	7.98**	1.42**	6.85**	6.03**	5.65**	4.09**	1.38**
800	12.35**	7.15**	8.86**	6.17**	1.44**	4.80**	3.25**	3.97**	2.83**	1.40**
$LSD_{5\%}$	1.88	1.72	2.23	1.49	0.07	1.83	1.20	1.66	1.51	0.05
$LSD_{1\%}$	2.58	2.36	3.06	2.04	0.10	2.51	1.64	2.27	2.07	0.07

SL: shoot lenght; RL: root length; SDM: shoot dry matter; RDM: root dry matter; IE: injurious effects; plants failed to survive * Significant (P = 0.05) and ** highly significant (P = 0.01) differences as compared with the control

The contents of Al, Ca, Mg and P in the main organs (root and shoot) of carrot and radish plants as affected by different levels of Al or Al-P interactions are given in Tables 2 and 3. The aluminium content increased in the main organs of the two test plants with increasing concentrations of Al alone (Tables 2 and 3). It is worth noting that the aluminium accumulation in radish was higher than in carrot. Also, in the Al treatments the contents of Ca, Mg and P in the two experimental plants showed a marked reduction in contrast to the control. On the other hand, the concentration of Al decreased in the main organs of the two test plants in the presence of phosphorus in the culture medium, as compared to the corresponding treatments with Al only. Generally, the Al contents were much higher in the case of radish than in carrot plants. The interactive effects of Al and P, shown in Table 2, led to the Ca content in carrot plants being more or less unaffected up to a level of 400 mol/L Al in the shoots and up to a level of 200 mol/Al in the roots. Thereafter, it was significantly reduced when compared with that of untreated plants, whichever plant organ was analysed. In most cases, phosphorus fertilization resulted in a highly significant increase in the contents of Mg and phosphate in the main organs of the two test plants, when compared with the corresponding levels of Al alone. This stimulatory effect was more obvious in carrot than in radish plants.

Table 2

Effect of Al stress and P fertilization on soluble sugars (mg/0.1 g frozen sample), soluble protein, total free amino acids, proline and mineral composition (mg g^{-1} dry matter) in shoots and roots of carrot plants

Treatments	Soluble sugar		Soluble protein		Free amino acids		Proline	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Al (mol/L)								
Control	19.83	23.70	45.8	34.6	9.58	6.76	1.45	1.85
200	20.17	24.27	47.3	35.8	8.75	5.03*	2.05**	2.11**
400	19.30	22.51	39.7**	30.1*	6.45**	4.41**	3.45**	3.67**
600	15.30**	18.33**	34.4**	28.7**	5.91**	4.13**	4.64**	3.74**
800	14.76**	15.50**	31.7**	25.3**	5.27**	3.94**	4.93**	3.53**
10 mM KH_2PO_4								
Control	22.70*	27.80*	56.8**	43.9**	10.75	7.26	1.27	1.59
200	22.50	27.92*	67.4**	49.2**	11.85*	8.52*	1.15	1.78
400	20.62	24.60	73.8**	53.2**	12.3**	10.60**	2.07**	1.97
600	16.24*	19.10**	77.5**	56.7**	14.80**	11.70**	2.21**	2.38**
800	15.33**	17.65**	80.2**	61.5**	14.05**	13.44**	2.88**	2.62**
$LSD_{5\%}$	2.73	3.17	5.12	3.62	1.81	1.67	0.33	0.29
$LSD_{1\%}$	3.74	4.34	7.01	4.96	2.48	2.29	0.44	0.40
	Al		Ca		Mg		P	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Al (mol/L)								
Control	0.51	0.80	9.30	12.28	7.55	6.16	5.88	4.75
200	0.90**	1.85**	7.07*	10.30*	7.03	4.05**	4.22**	4.08**
400	1.14**	2.40**	6.73**	8.20**	6.25**	3.45**	2.12**	2.75**
600	1.67**	2.85**	5.15**	7.35**	4.17**	3.65**	1.75**	1.13**
800	2.08**	3.16**	4.77**	5.31**	3.74**	2.18**	1.77**	1.22**
10 mM KH_2PO_4								
Control	0.42	0.63	11.22*	14.55*	8.16**	6.80**	7.15**	6.08**
200	0.61	0.96	10.65	13.35	7.85	5.79*	6.02	5.85**
400	0.70*	1.08*	9.03	9.57**	7.18	5.05**	5.70	5.30*
600	1.24**	1.83**	6.78**	9.23**	6.81	4.23**	4.85**	3.66**
800	1.77**	2.05**	6.04**	8.28**	5.45**	3.86**	2.83**	2.49**
$LSD_{5\%}$	0.16	0.21	1.77	1.81	0.83	0.39	0.32	0.28
$LSD_{1\%}$	0.22	0.29	2.42	2.48	1.14	0.53	0.44	0.38

* Significant ($P = 0.05$) and ** highly significant ($P = 0.01$) differences as compared with the control

Discussion

The harmful effects of Al toxicity on the two experimental plants in the present work were clearly manifested in the significant reduction in root elongation and dry matter production. In addition, the higher shoot/root ratio was a result of the greater reduction in root growth than that of the shoot. This reduction was greater for radish than for carrot. Similar results were also obtained by Jorge and Arruda (1997) and Gaume et al. (2001). On the other hand, when P fertilizer was applied to the tested plants, the harmful effects of Al toxicity were significantly decreased, especially at low and moderate levels of

Table 3

Effect of Al stress and P fertilization on soluble sugars (mg/0.1 frozen sample), soluble protein, total free amino acids, proline and mineral composition (mg g^{-1} dry matter) in shoots and roots of radish plants

Treatments	Soluble sugar		Soluble protein		Free amino acids		Proline	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Al (mol/L)								
Control	15.85	19.20	31.2	23.7	19.0	22.7	3.13	1.66
200	13.75*	17.44**	37.7*	26.9*	16.8*	17.41**	5.23**	2.27**
400	11.3**	14.27**	39.8**	29.9**	12.65**	16.50**	5.88**	3.97**
600	9.55**	13.65**	45.5**	33.4**	12.10**	14.23**	6.72**	5.38**
800	IE	IE	IE	IE	IE	IE	IE	IE
10 mM KH_2PO_4								
Control	16.25	20.80	39.9**	28.9**	20.77**	23.12*	3.72*	2.15**
200	15.38	19.94	41.5**	33.7**	19.39**	24.43**	3.56	2.80**
400	13.74*	17.37*	44.9**	35.9**	22.05**	26.25*	4.62**	3.15**
600	12.12**	16.50**	49.5**	39.9**	23.91**	26.11*	5.07**	4.25**
800	10.46**	13.27**	52.6**	43.7**	26.75**	27.35**	5.94**	5.75**
$LSD_{5\%}$	1.85	1.42	5.07	3.01	1.77	2.92	0.53	0.25
$LSD_{1\%}$	2.50	1.92	7.00	4.12	2.39	4.00	0.72	0.34
	Al		Ca		Mg		P	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Al (mol/L)								
Control	0.71	0.86	7.55	10.88	7.02	6.40	5.55	4.47
200	1.22**	2.07**	6.30**	9.09**	6.64	5.90	4.47**	3.03**
400	1.65**	3.54**	4.74**	6.65**	5.32**	3.36**	2.10**	1.25**
600	2.83**	4.27**	4.23**	5.77**	3.88**	1.88**	1.27**	1.12**
800	IE	IE	IE	IE	IE	IE	IE	IE
10 mM KH_2PO_4								
Control	0.67	0.77	11.10**	12.05**	7.60	8.36**	6.80**	5.15**
200	0.86	1.17	9.50**	11.07**	6.84	5.64*	5.77	4.88*
400	1.35**	2.24**	6.60**	7.55**	6.22*	4.33**	5.05	4.10*
600	2.32**	3.18**	5.42**	5.04**	5.63**	2.79**	3.06**	2.70**
800	3.63**	4.23**	5.33**	4.88**	3.71**	1.93**	3.11**	2.17**
$LSD_{5\%}$	0.19	0.34	0.91	1.07	0.69	0.67	0.54	0.32
$LSD_{1\%}$	0.26	0.46	1.25	1.47	0.94	0.90	0.74	0.44

* Significant (P = 0.05) and ** highly significant (P = 0.01) differences as compared with the control. IE: Injurious effects; plants failed to survive

Al (Gaume et al., 2001; Nakagawa et al., 2003). This suggests that P application may act as a growth stimulant, which can play a role in reversing the effect of Al toxicity on the metabolic activities relevant to growth by enhancing cell division and/or cell enlargement. This would result in longer shoots and roots with a consequent increase in dry matter production.

In the present study, the soluble sugars in the carrot plants remained more or less unchanged at low and moderate levels of Al. Thereabove, the content of soluble sugars sharply decreased in response to Al toxicity. This is in agreement with the results obtained by Kochian (1995) and Heim et al. (1999). In radish

plants, aluminium induced a progressive decrease in the amount of soluble sugars, whichever plant organ was analysed. The inhibition of carbohydrate biosynthesis recorded in the two test plants is harmful because of its effect on the potential gradient between shoots and roots which helps in water absorption and translocation. In this study, soluble protein remained more or less unchanged at low levels of Al. However, with higher Al treatments, somewhat different effects were recorded. In radish the losses in soluble sugars were accompanied by increases in soluble protein, while in carrot plants the opposite effect was observed. This suggests that Al tolerance is linked with an equilibrium and interconversion between soluble sugars and the nitrogen metabolism, whereas Al toxicity leads to metabolic disturbances in both components. This agrees with the results obtained by Taylor (1988; 1991). On the other hand, phosphorus application generally stimulated the accumulation of organic solutes (soluble sugars and soluble protein) in the various Al-affected organs in the two test plants and the inhibitory effects of Al toxicity were partially or completely alleviated.

The pattern of changes in amino acids was opposite to that of proline, indicating that the increase in proline is at the expense of other amino acids through an effect of aluminium in promoting their conversion in the two test plants. These results are in agreement with those obtained by Galvez et al. (1991) and Zaifnejad et al. (1997). Therefore, it may be concluded that the accumulation of proline is one of the major physiological mechanisms of Al tolerance in the experimental plants. Under the influence of the Al-P interaction, the accumulation of proline was considerably retarded whichever plant organ was analysed. This reduction in proline accumulation was concomitant with the promotion in growth of the two test plants, suggesting that the addition of P can alleviate the toxicity of Al (Alva, 1986; de Miranda and Rowell, 1987; Nakagawa et al., 2003).

The accumulation of Al was greatly elevated at higher Al levels in the main organs of the test plants used in this work. In this respect, the root systems of the two test plants accumulated higher amounts of Al than the shoot systems. This is in accordance with the results obtained by Amancio et al. (1991) and Gaume et al. (2001). The content of Al in the main organs decreased in both plants when P was supplied. Therefore, it can be said that additions of P fertilizer to the soil could decrease Al toxicity, due to the direct precipitation of Al-phosphates in the zone of P incorporation. In addition, it has been shown that soluble, yet non-toxic Al-P complexes may form in solution (Alva, 1986). Also, the Ca, Mg and P contents in the two test plants decreased, especially in the roots, as also reported by other authors (Rost-Siebert, 1983; Hahn and Marschner, 1998; Gaume et al., 2001). After fertilization with phosphorus, the Ca, Mg and P contents of the main organs in the two test plants were significantly elevated. These effects confirm the results obtained by McLaughlin and James (1991), Taylor (1991) and Gaume et al. (2001), who reported that P

exerted a slight effect in counteracting the adverse effect of aluminium toxicity. Also, in most soils, surface-applied P has been found to improve root penetration into acidic subsoils (de Miranda and Rowell, 1987). In addition, McLaughlin and James (1991) postulated that P nutrition may affect resistance to Al through changes in the cation-anion balance within plants. Generally, it can be said that the beneficial effect of P was reflected in the reduction of aluminium accumulation and/or the increase in the concentration of Ca, Mg and P.

Finally, it can be seen that P fertilization generally alleviated the harmful effects of Al toxicity in carrot and radish plants, especially at low and moderate levels of Al stress. This was accompanied by increases in soluble sugars and soluble protein, which were always associated with an increase in the root elongation and dry matter production of the test plants. This would suggest that the depressive effects of Al stress on seedling growth and other relevant physiological activities can be alleviated and/or regulated to some extent by P fertilization. In addition, carrot plants exhibited greater tolerance to Al stress than radish plants.

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Acta Agronomica Hungarica, 53(3), pp. 303–308 (2005)

RELATIVE EFFICACY OF VERMICOMPOST AND FARMYARD MANURE INTEGRATED WITH INORGANIC FERTILIZERS FOR SUSTAINABLE PRODUCTIVITY OF FORAGE SORGHUM (*SORGHUM BICOLOR* (L.) MOENCH)

R. S. SHEORAN and D. S. RANA

FORAGE RESEARCH SECTION, CCS HARYANA AGRICULTURAL UNIVERSITY, HISAR, INDIA

Received: 24 August, 2004; accepted: 18 March, 2005

A field experiment was carried out on sandy loam soils at CCS Haryana Agricultural University, Hisar, India during the summer/rainy season from 1998–99 to 2000–01. The experiment consisted of ten fertility treatments having control, 50, 75 and 100% recommended dose of fertilizers, vermicompost and farmyard manure alone and in combination with chemical fertilizers. The three-year study revealed that the application of 10 t/ha vermicompost or farmyard manure each along with 75% recommended doses of fertilizers gave significantly higher green fodder and dry matter yield than all other treatments except 100% recommended dose of fertilizers. Likewise, the fodder yield obtained from the use of 10 t/ha vermicompost + 50% recommended dose of fertilizers was found to be statistically at par with that obtained from the application of 75% recommended dose of fertilizers alone, indicating that 25% chemical fertilizers could be substituted. The application of 10 t/ha vermicompost along with 75% recommended dose of fertilizers increased the green fodder and dry matter yield by 60.8 and 66.2%, respectively, over the control on a pooled mean basis. Non-significant differences were observed between the organic manures for forage yield, though the application of vermicompost gave a slightly higher yield than farmyard manure.

Key words: vermicompost, farmyard manure, inorganic fertilizer, sorghum, forage yield, productivity

Introduction

During the last three decades, chemical fertilizers have been the major source of nitrogen supply to field crops. In the recent past, these chemical fertilizers have been observed to have adverse effects in terms of soil health and a decline in productivity (Sharma et al., 1994). The results of a large number of experiments on manures and fertilizers conducted in India and abroad revealed that neither chemical fertilizers alone nor organic sources alone could achieve production sustainability for either soil or crops in intensive cropping systems. Later, the concept of integrated nutrient supply involving the use of organic manures gained importance in the nutrient management of crop production (Nambiar and Abrol, 1989). Farmyard manure is a major source of organic nutrition for field crops, but the limited availability of this manure is a constraint on its use. In recent years, vermicompost has been advocated as a good organic manure for use in integrated nutrient management systems in field crops (Shroff and Devasthali, 1992; Bhawalkar, 1992).

Sorghum is one of the most important cereal fodder crops grown extensively during the summer (rainy) season in India. Due to its excellent growing habit, quick re-growth and good nutritive value, it is greatly favoured by farmers. It is admirably adapted to varying soils and climatic conditions. A growing body of evidence indicates that sorghum responds well to applied nitrogen (Patel et al., 1990; Tomar and Agarwal, 1993; Patel, 1998; Desale et al., 1999). Various research workers have reported a positive response of cereal forages to applied nitrogen in conjunction with various organic manures. However, the information available on the effect of vermicompost and on nutrient substitution through this manure in forage sorghum is meagre. Therefore, an attempt has been made to study the effect of the integration of chemical nitrogen with vermicompost on forage sorghum for sustained production.

Materials and methods

A field experiment was conducted at the Forage Research Farm of CCS Haryana Agricultural University, Hisar, India during the summer/rainy season for three consecutive years from 1998–99 to 2000–01 on forage sorghum under semi-arid conditions. The experiment consisted of ten fertility treatments, namely control (T_1), 50% recommended dose of fertilizers (RDF) (T_2), 75% RDF (T_3), 100% RDF (T_4), 10 t/ha vermicompost (T_5), 10 t/ha farmyard manure (FYM) (T_6), 50% RDF + 10 t/ha vermicompost (T_7), 50% RDF + 10 t/ha FYM (T_8), 75% RDF + 10 t/ha vermicompost (T_9) and 75% RDF + 10 t/ha FYM (T_{10}). All these treatments were replicated thrice in a randomized block design.

The soil of the experimental site was analysed for various physical and chemical properties. The soil was sandy loam (Typic Ustochrepts) in texture, low in organic carbon and available nitrogen, medium in available phosphorus, high in available potash and slightly alkaline in reaction, having pH 7.9 (Table 1). The available nitrogen in the soil was estimated by the alkaline permanganate method (Subbiah and Asija, 1956), available phosphorus was determined using 0.5 M $NaHCO_3$ (pH 8.5) extractant (Olsen et al., 1954) and available potassium was determined with the neutral normal ammonium acetate method (Jackson, 1967).

Since the soil was rich in available potash, only the recommended dose of nitrogen (80 kg N/ha) and phosphorus (20 kg P_2O_5/ha) was applied in treatments of N and P. The full dose of phosphorus in the form of single superphosphate was drilled into the soil prior to sowing, whereas half the nitrogen was applied as urea at the time of sowing and the remaining half was top dressed one month after sowing. The chemical composition of vermicompost and farmyard manure is given in Table 2, which indicates the total amount (organic + inorganic form) of the nutrients in the vermicompost or FYM.

Table 1
Chemical composition of soil before sowing (0–15 cm soil depth)

Year	Organic carbon (%)	pH	Ece (dS m^{-1} at 25°C)	Available		
				N (kg/ha)	P (kg/ha)	K (kg/ha)
1998–1999	0.39	7.9	0.24	164.0	15.2	352.2
1999–2000	0.40	8.0	0.28	179.2	16.5	331.0
2000–2001	0.35	7.9	0.21	169.2	17.2	368.4

Table 2
Chemical composition of vermicompost and farmyard manure

Contents	Vermicompost			Farmyard manure		
	1998–99	1999–00	2000–01	1998–99	1999–00	2000–01
Moisture (%)	58.34	63.20	61.0	56.30	58.24	55.50
Nitrogen (%)	1.50	1.44	1.40	0.81	0.85	0.79
Phosphorus (%)	1.05	1.10	1.09	0.35	0.34	0.33
Potash (%)	1.90	1.96	1.81	0.85	0.86	0.92

The organic manures were mixed well into the soil about three weeks before sowing. Soil samples for chemical analysis were taken prior to the application of organic manures. The sorghum variety HC-171 was sown in lines 30 cm apart on 4 July, 7 July and 25 June in 1998–99, 1999–2000 and 2000–01, respectively, using a seed rate of 40 kg/ha. To keep the crop free of weeds, inter-culture operations were carried out as per recommendations. Irrigation was applied as and when required. The crop was harvested at the 50% flowering stage. The data on fodder yield and other yield attributes were recorded at the time of harvesting. A green plant sample of 500 g was taken from each treatment and sun dried. These samples were then oven dried at 60°C for 24 hours to estimate the dry matter content.

Results and discussion

The three-year study revealed that all the levels of chemical fertilizer and both the sources of organic manure alone or in combination with various doses of chemical fertilizers significantly improved the forage yield over the control in the individual years and also over pooled analysis (Table 3).

Table 3
Effect of various fertility levels on green fodder and dry matter yields (t/ha) of sorghum

Treatments	Green fodder yield				Dry matter yield			
	1998–99	1999–00	2000–01	Pooled	1998–99	1999–00	2000–01	Pooled
T_1	28.7	29.7	34.0	30.8	7.1	7.4	8.6	7.7
T_2	31.5	40.2	41.7	37.8	8.2	10.2	10.0	9.5
T_3	33.0	45.0	47.8	41.9	8.3	12.1	11.6	10.7
T_4	36.3	52.2	55.0	47.8	8.9	13.6	13.1	11.8
T_5	33.2	41.2	45.8	40.0	8.5	11.1	11.3	10.3
T_6	31.9	40.8	43.8	38.8	8.1	11.3	10.7	10.0
T_7	35.4	47.8	50.7	44.6	9.0	12.9	12.7	11.5
T_8	33.7	46.7	49.2	43.2	8.4	12.5	11.8	10.9
T_9	37.4	53.7	57.5	49.5	9.2	15.0	14.1	12.8
T_{10}	34.7	51.7	55.3	47.2	8.6	13.8	13.4	11.9
CD (5%)	2.4	4.9	5.3	3.9	0.4	1.7	1.9	1.0

T_1 = Control; T_2 = 50% RDF; T_3 = 75% RDF; T_4 = 100% RDF; T_5 = 10 t/ha Vermicompost; T_6 = 10 t/ha FYM; T_7 = 50% RDF + 10 t/ha Vermicompost; T_8 = 50% RDF + 10 t/ha FYM; T_9 = 75% RDF + 10 t/ha Vermicompost; T_{10} = 75% RDF + 10 t/ha FYM; RDF: Recommended dose of fertilizers; FYM: Farmyard manure

Table 4
Effect of various fertility levels on growth parameters of sorghum

Treatments	Plant height (cm)				No. of tillers per metre row length			
	1998–99	1999–00	2000–01	Pooled	1998–99	1999–00	2000–01	Pooled
T_1	167.0	206.8	203.2	192.3	21.1	20.0	23.4	21.5
T_2	205.2	235.5	269.2	236.6	24.8	23.3	28.0	25.4
T_3	220.0	281.1	290.1	263.7	26.1	24.6	30.1	26.9
T_4	242.8	301.1	310.0	284.6	27.9	25.8	31.5	28.4
T_5	225.1	267.8	281.1	258.0	27.4	25.9	29.4	27.6
T_6	218.0	250.6	275.3	248.0	25.6	24.9	29.0	26.5
T_7	234.4	288.0	301.1	274.5	27.5	25.8	30.8	28.0
T_8	227.6	283.7	298.1	269.8	26.2	25.4	30.5	27.4
T_9	259.5	302.5	319.1	293.7	28.0	26.9	32.8	29.2
T_{10}	240.1	296.5	318.0	284.9	27.4	26.1	32.4	28.6
CD (5%)	24.2	20.7	23.1	23.4	2.6	3.2	3.5	3.3

T_1 = Control; T_2 = 50% RDF; T_3 = 75% RDF; T_4 = 100% RDF; T_5 = 10 t/ha Vermicompost; T_6 = 10 t/ha FYM; T_7 = 50% RDF + 10 t/ha Vermicompost; T_8 = 50% RDF + 10 t/ha FYM; T_9 = 75% RDF + 10 t/ha Vermicompost; T_{10} = 75% RDF + 10 t/ha FYM; RDF: Recommended dose of fertilizers; FYM: Farmyard manure

Increasing the quantity of fertilizer from 50 to 100% of the recommended dose resulted in a significant increase in the forage yield over the preceding dose. An increase of 22.7, 36.2 and 55.3% for green fodder and 22.8, 38.9 and 53.9% for dry matter yield was recorded over the control with the application of 50, 75 and 100% of the recommended dose, respectively, averaged over three years. Such results are to be expected, because nitrogen and phosphorus are essential elements for the growth and development of plants, which is clearly reflected through the increased plant height and greater number of tillers per metre row length with increasing levels of nitrogen and phosphorus compared with the control and lower doses (Table 4). These results corroborate the findings reported by Kumar and Sharma (1990), Sadhu et al. (1991), Tomar and Agarwal (1993), Patel (1998) and Desale et al. (1999). Increasing levels of recommended fertilizers reduced the days to 50% flowering, which ultimately increased the productivity of the crop (Table 5).

The application of organic manures, i.e. 10 t/ha vermicompost or FYM alone, significantly increased the fodder yield over the control treatment. The average increase in green fodder yield with the application of 10 t/ha vermicompost or FYM compared with the control was 30.1 and 26.1%, respectively, while the corresponding values for dry matter yield were 33.6 and 30.2%, respectively. Non-significant differences were observed between the organic manures for forage yield. However, the application of vermicompost gave a slightly higher yield than FYM. The organic manures not only supplied available nutrients to the plants but also had solubilizing effects on the fixed forms of other nutrients (Sinha et al., 1981), therefore improving the productivity of the crop. Vermicompost had a direct effect by supplying

Table 5
Effect of various fertility levels on flowering and productivity of sorghum

Treatments	Days to 50 % flowering				kg green fodder/day/ha				kg dry matter/day/ha			
	1998–99	1999–00	2000–01	Pooled	1998–99	1999–00	2000–01	Pooled	1998–99	1999–00	2000–01	Pooled
T_1	82.0	81.0	79.0	80.7	350.2	366.2	430.4	382.2	86.1	91.5	108.9	95.5
T_2	76.6	77.4	75.7	76.5	411.3	519.0	550.4	493.5	106.9	131.5	132.1	123.5
T_3	74.0	74.1	73.0	73.7	445.8	607.3	655.2	569.4	112.7	163.6	158.5	144.9
T_4	69.3	69.5	69.0	69.3	524.1	750.5	797.1	690.5	128.1	195.1	189.5	170.9
T_5	74.1	73.8	72.0	73.3	447.6	557.8	636.5	547.3	114.2	150.4	156.2	140.3
T_6	73.9	74.0	73.2	73.1	431.3	551.7	598.8	527.3	109.5	153.1	145.5	136.0
T_7	71.8	72.2	71.0	71.7	493.0	662.4	713.7	623.0	124.9	178.9	178.5	160.8
T_8	72.1	74.0	71.5	72.5	467.8	630.6	687.6	595.3	116.9	169.3	165.3	150.5
T_9	68.0	68.3	67.1	67.8	550.5	785.8	856.9	731.1	135.1	220.0	210.6	188.6
T_{10}	69.0	70.8	67.8	69.2	502.3	729.6	815.6	682.5	124.3	194.2	197.2	171.9
CD (5%)	4.6	5.2	5.0	4.8	49.5	58.1	54.0	53.1	12.1	20.0	16.4	17.9

T_1 = Control; T_2 = 50% RDF; T_3 = 75% RDF; T_4 = 100% RDF; T_5 = 10 t/ha Vermicompost; T_6 = 10 t/ha FYM; T_7 = 50% RDF + 10 t/ha Vermicompost; T_8 = 50% RDF + 10 t/ha FYM; T_9 = 75% RDF + 10 t/ha Vermicompost; T_{10} = 75% RDF + 10 t/ha FYM; RDF: Recommended dose of fertilizers; FYM: Farmyard manure

essential nutrients and an indirect effect by introducing earthworms into the field. Vermicompost has been reported to contain a large number of nitrogen-fixing, phosphate-solubilizing bacteria and other beneficial microbes, antibiotics, vitamins, hormones, enzymes, etc., which have a favourable effect on the growth and yield of plants (Bhawalkar, 1992). All these factors made vermicompost a better manure than FYM for the sustained productivity of sorghum. Shroff and Devasthali (1992) and Singh et al. (1998) also reported a comparatively better response of field crops to vermicompost.

The integration of chemical fertilizers with both the organic manures also exerted favourable effects on the fodder production of sorghum. The application of 10 t/ha vermicompost + 75% RDF gave significantly higher green (49.5 t/ha) and dry matter (12.7 t/ha) yields than all the treatments under test except the treatment having 10 t/ha FYM + 75% RDF (47.2 t/ha green fodder and 11.9 t/ha dry matter) on a three-year average. This combination of 10 t/ha vermicompost with 75% RDF also remained statistically at par with 100% RDF, indicating that the substitution of 25% recommended dose of chemical fertilizers is possible with this treatment. Similarly, the forage yield obtained from the use of vermicompost + 50% RDF was statistically at par with that obtained from 75% RDF alone, again showing the possible replacement of 25% chemical fertilizer.

The application of vermicompost along with 75% RDF increased the green fodder and dry matter yield by 60.8 and 66.2%, respectively, over the control on a pooled mean basis. This increase in forage yield with the integration of vermicompost and chemical fertilizers can be attributed to the increased growth parameters (Table 4) as well as to the productivity of the crop (Table 5). The combination of organic manures and chemical fertilizers also improved the

productivity of the crop, which is mainly due to the reduction in the days to 50% flowering. Vasanthi et al. (1998) and Jayanthi et al. (2002) also reported the favourable effect of integrating organic manures and chemical fertilizers on the forage yield of various crops.

Hence, it is concluded that the integrated use of organic manures along with inorganic fertilizers may be advocated for the partial replacement of the chemical fertilizers as well as for obtaining a higher yield of forage sorghum.

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Acta Agronomica Hungarica, 53(3), pp. 309–317 (2005)

EFFECTS OF SEEDING DENSITIES AND ROW SPACING ON YIELD AND YIELD COMPONENTS OF LINSEED (*LINUM USITATISSIMUM* L.)

F. U. HASSAN[1], M. H. LEITCH[2] and M. K. ABBASI[3]

[1]DEPT. OF AGRONOMY, UNIVERSITY OF ARID AGRICULTURE, RAWALPINDI, PAKISTAN
[2]UNIVERSITY OF WALES, ABERYSTWYTH, CEREDIGION, UK
[3]UNIVERSITY COLLEGE OF AGRICULTURE, RAWALAKOT, AJK, PAKISTAN

Received: 23 September, 2004; accepted: 31 July, 2005

The space available to plants affects the available resources and hence modifies the growth habits and yield potential of plants. The effect of four seeding densities (250, 500, 750 and 1000 viable seeds/m^2) and three row spacings (12, 15 and 20 cm) were evaluated at Morfa Mawr field station at the University of Wales, Aberystwyth, UK during the 1993 and 1994 growing seasons. Number of capsules per plant, number of seeds per capsule, thousand-seed weight and seed yield were examined during the study. Capsule index (CI) and harvest index (HI) were calculated from the observed data. During the first year, capsules per plant, seeds per capsule and capsule index were increased by decreasing the seed density, while the maximum seed yield of 3.9 t/ha was recorded at the highest seed density of 1000 seeds/m^2. An increase in row spacing led to an almost linear increase in most of the yield attributes of the crop. During the second year, the response of yield and yield attributes to seeding densities was similar to that recorded during the first year. Seed yield increased with decreasing row spacing, while the rest of the components did not show any consistent response. During the first year, the overall performance and production of the crop was higher than in the second year because of the better weather conditions, with mild temperature and high rainfall during the season.

Key words: *Linum usitatissimum*, linseed, seeding densities, row spacing, yield components

Introduction

Plant population or plant density is defined as the number of plants per unit area. Optimum populations obviously vary considerably with crop species, depending largely upon the morphological structure of the plant, environmental factors and the end product. Crops such as maize and sugar beet are simple structures based on a single stem, thus the individual plant represents a single unit of population, but cereal plants tend to produce tillers which will contribute to yield. In this case, the number of tillers per unit area, rather than plants per unit area may be a more meaningful measure of plant 'population'. Crop yield is affected not only by pests and diseases, but also by the effects of neighbouring plants. The competition of plants with their neighbours reflects a struggle for the resources available within the production environment. The physical resources in the environment are usually water (moisture availability), soil nutrients and light. Where the supply of one of these parameters is reduced to a sub-optimal level, plant growth and hence crop yield may be reduced (Clipson and Edwards, 1994).

0238–0161/$ 20.00

The growth habits and yield potential of plants are modified as a result of differences in available space and hence differences in the resources available for their development (Bilgili et al., 2003). An optimum population utilizes the available nutrients, soil moisture and light more efficiently, otherwise these resources remain under-utilized. However, at a given level of plant population, it is possible to vary the spacing in order to obtain a good canopy with almost uniform illumination of leaves throughout its profile, thereby creating conditions in which plants will be complementary to each other in fully exploiting the available resources rather than competing with each other (Hassan and Leitch, 2001). The development of a well-structured and receptive canopy is dependent upon the total population of plants in a unit area. A greater number of plants will result in less space available for their development. Within the environment of densely packed plants, roots will find less volume of soil for moisture and nutrient availability. Under such conditions of higher population and closer spacing, plants grow taller and leaf density increases (Albrechtsen and Dybing, 1973). The lower leaves become shaded by the upper leaves, reducing the photosynthetic efficiency of the lower leaves and ultimately of the whole plant. Generally plants have the ability to respond to spacing by producing secondary shoots/tillers to fill the available space, which is why the yield per unit area remains relatively constant over a range of densities (Clipson and Edwards, 1994).

The response to available space and density is also related to species, varieties and purpose of growth. When *Linum usitatissimum* is grown for fibre (flax) it is usually planted at much higher densities than when it is grown for oil (linseed), since the presence of secondary shoots reduces fibre quality. However, for maximum seed yield it is necessary to choose a population density which avoids the inefficient use of resources at low density and excessive competition at higher densities. Since linseed is normally sown in rows or drills, the precise arrangement of the seed is influenced not only by the seed density but also by row spacing. The specific densities can be arranged in a different fashion as row spacing differs.

The present study was designed to investigate the effect on the yield and yield components of sowing a commercial linseed cultivar at different seed densities with different row spacings.

Materials and methods

The experiments were conducted at the Morfa Mawr field station of the University of Wales, Aberystwyth, some 20 km south of Aberystwyth, on a deep sandy loam soil belonging to the Cegin series, during 1993 and 1994. Prior to the experiments the respective fields were grazed as a temporary ley for three years. The experimental area was ploughed in September 1992 and 1993. Potassium and phosphorus fertilizers were incorporated in March 1993 and 1994 at a rate equivalent to 250 kg/ha of K_2O and P_2O_5. The regrowth was sprayed with Paraquat (ICI, London, UK: Gramoxone) prior to final seedbed preparation. The experiments were laid out in a two-factor randomized block design having all combinations of four seed densities (250, 500, 750 and 1000 seeds/m^2) and three row spacings (12, 15 and 20 cm) with four replications.

The cultivar Antares was drilled at the appropriate seed densities on 28th April 1993 and 4th May 1994 with an Oyjord drill (Germany). The plots were 8 m × 1.2 m and consisted of 10, 8 and 6 rows, respectively, at the 12, 15 and 20 cm row spacings. Nitrogen was applied at a rate equivalent to 40 kg/ha by hand immediately after drilling. The plots were sprayed with Bentazone (ICI, London, UK: Basogran) on 5th June 1993 and 11th June 1994 to control broadleaf weeds. Subsequently, weeds were controlled by hand when required.

An area one metre in length from two central rows of each plot was harvested on 14th September 1993 and on 8th September 1994. The harvested plants were laid out to air dry in an open glasshouse for two weeks. The total number of plants and shoots was counted in each sample. A sub-sample of ten plants was taken at random in order to measure the yield components. The total number of capsules per plant was counted before separation. The separated capsules were threshed by hand and the total number of seeds counted. The bulk samples were threshed with a small grain thresher, especially modified for linseed. The seeds were cleaned by blowing with a hair drier, then weighed, and the yield per hectare was calculated from this bulk sample. Three lots of one thousand seeds were counted and weighed for thousand-seed weight. Capsule index and harvest index were calculated from the collected data of capsule dry weight, total biomass and total seed yield. The collected data were analysed using standard analysis of variance techniques.

Results

In both the years (1993 and 1994) the total number of capsules per plant decreased as seed density was increased (Fig. 1a). However, in 1993 the number of capsules per plant decreased significantly as the seed density increased progressively up to 750 seeds/m^2, after which there was no significant decrease (Table 1). In 1994 the lowest (250) seed density produced a significantly greater number of capsules per plant than all other treatments. The differences between the mid-range densities and between the two highest densities were not statistically significant (Table 2). Again the lowest (250) and the highest (1000) seed densities gave the greatest and smallest numbers of capsules per plant, respectively, in both of the experiments. In 1993 and 1994, although the capsules per plant decreased as the row spacing decreased (Tables 1 and 2; Fig. 1b), the differences were not significant. In addition, there was no significant interaction between the effects of seed density and row spacing on the number of capsules per plant.

Table 1
Effects of seed density and row spacing on yield and yield components in 1993

Seed density (seeds/m^2)	Capsules per plant	Seeds per capsule	TSW (g)	Seed yield (t/ha)	CI (%)	Harvest index (%)
250	37.68	7.73	7.93	3.59	78.01	37.47
500	21.51	7.36	8.08	3.86	65.75	36.80
750	14.83	7.31	7.81	3.84	61.76	37.41
1000	13.48	7.16	8.02	3.90	57.89	39.46
SE	1.523	0.198	0.127	0.223	2.876	1.897
Row spacing (cm)	Capsules per plant	Seeds per capsule	TSW (g)	Seed yield (t/ha)	CI (%)	Harvest index (%)
20	23.88	7.33	7.98	4.07	69.54	41.01
15	20.14	7.60	7.92	3.97	65.38	37.75
12	21.61	7.23	7.98	3.35	62.64	34.59
SE	1.319	0.172	0.110	0.193	2.491	1.643

Table 2
Effects of seed density and row spacing on yield and yield components in 1994

Seed density (seeds/m^2)	Capsules per plant	Seeds per capsule	TSW (g)	Seed yield (t/ha)	CI (%)	Harvest index (%)
250	20.9	7.6	8.44	3.16	113.1	51.5
500	12.1	7.2	8.01	3.42	107.1	50.2
750	10.1	7.1	7.79	3.73	102.4	50.4
1000	8.4	7.2	7.64	3.96	101.1	52.5
SE	0.71	0.09	0.120	0.251	2.58	3.38
Row spacing (cm)	Capsules per plant	Seeds per capsule	TSW (g)	Seed yield (t/ha)	CI (%)	Harvest index (%)
20	12.6	7.3	7.98	3.41	107.3	50.6
15	13.3	7.2	7.99	3.61	106.9	50.5
12	12.7	7.3	7.94	3.68	103.6	52.4
SE	0.61	0.07	0.110	0.217	2.24	2.92

The number of seeds per capsule decreased as seed density increased progressively in 1993. The maximum and minimum number of seeds per capsule were recorded from the lowest (250) and the highest (1000) seed densities, respectively, which were the only treatments found to be significantly different (Table 1, Fig. 2a). In 1994, though the number of seeds per capsule decreased with the increase in seed density, the decrease was not progressive or consistent. The number of seeds per capsule decreased as the seed density increased up to 750 seeds/m^2, then showed a small increase at the highest (1000) density. The number of seeds per capsule in the 250 seeds/m^2 treatment was significantly greater than in the other density treatments (Table 2). There were no consistent effects of row spacing on seeds per capsule, but contrasting effects in the two years (Tables 1 and 2; Fig. 2b). The interaction between the effects of seed density and row spacing remained statistically non-significant on the number of seeds per capsule in both the years.

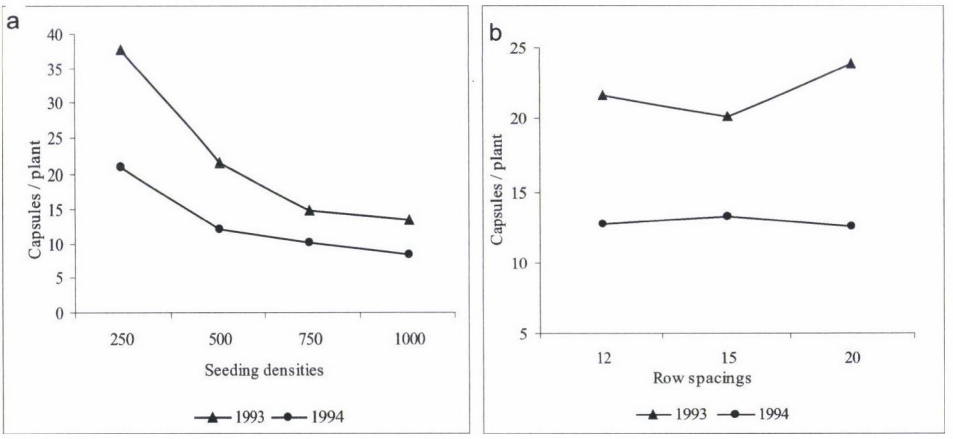

Fig. 1. Effects of (a) seeding density and (b) row spacing on capsules/plant

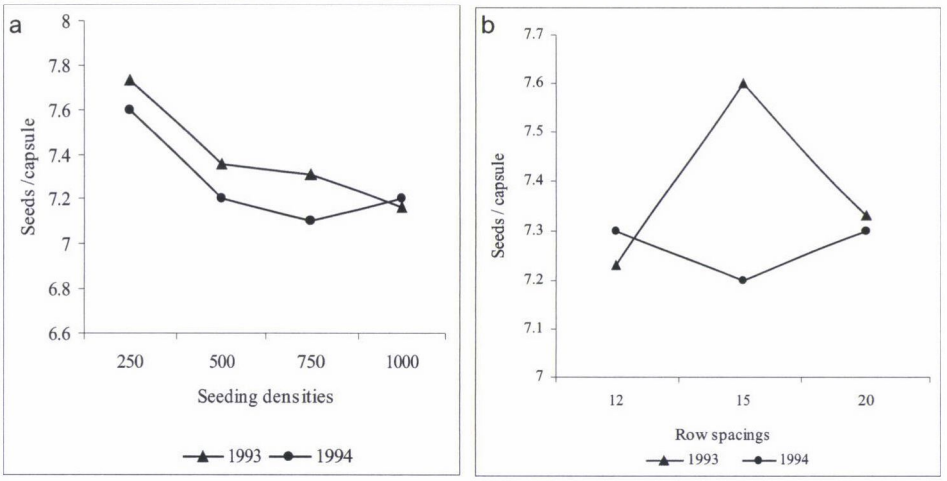

Fig. 2. Effects of (a) seeding density and (b) row spacing on seeds/capsule

Thousand-seed weight (TSW) was not affected in any consistent pattern and differences remained statistically non-significant between any of the treatments tested in 1993 (Table 1). However, in 1994, TSW decreased as seed density increased (Fig. 3a). The TSW of the lowest (250) seed density was significantly greater than that of the other density treatments (Table 2). The differences between the mid-range densities and between the two highest densities were not statistically significant. In both the experiments the effects of row spacing on TSW were found to be interesting (Tables 1 and 2; Fig. 3b), but statistically non-significant. The interaction between the effects of seed density and row spacing on TSW was not statistically significant either.

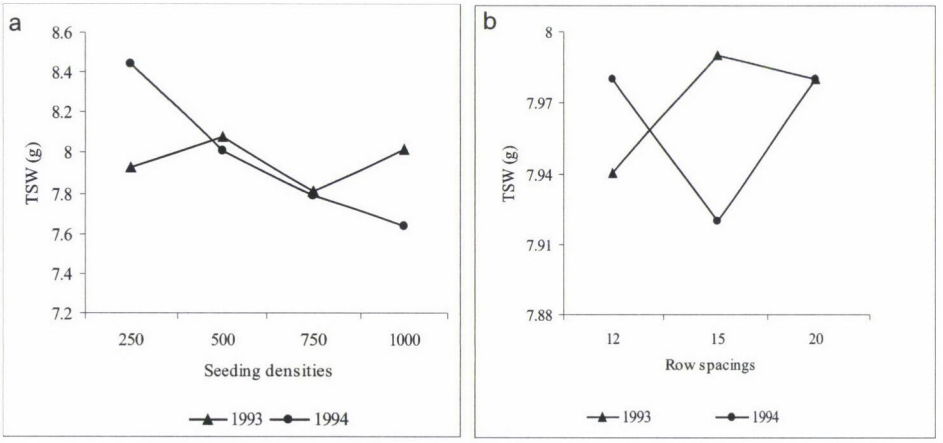

Fig. 3. Effects of (a) seeding density and (b) row spacing on TSW (g)

Seed yield per unit area (t/ha) increased as seed density increased (Fig. 4a). The largest and smallest seed yields were obtained from the highest (1000) and the lowest (250) seed densities, respectively, but there were no significant differences between the seed densities in 1993 (Table 1). In 1994, however, the highest (1000) seed density gave a significantly greater seed yield than the lowest (250) seed density, while the differences between the remaining treatments were not statistically significant (Table 2). The response of the seed yield to row spacing was contrary in the two years. In 1993, the seed yield increased with the row spacing. In 1994, however, the seed yield decreased as the row spacing increased (Tables 1 and 2; Fig. 4b). There was no significant interaction between the effects of seed density and row spacing on seed yield.

In both the experiments (1993 and 1994) the ratio of capsule weight to stem weight (capsule index, CI) decreased progressively as the seed density increased. The lowest (250) seed density gave a significantly greater CI than the higher densities, which were not significantly different (Tables 1 and 2). The effects of row spacing on CI were found to be consistent in both the years (Tables 1 and 2). However, CI was greater in 1994 as compared to 1993.

The ratio of seed yield to biomass (harvest index, HI) was not affected in any consistent pattern by seed densities in either of the experiments (Tables 1 and 2). However, HI increased progressively as row spacing increased in 1993. The widest (20 cm) row spacing gave a significantly greater HI than the closest (12 cm) row spacing, while HI decreased as row spacing was increased in 1994 and the closest (12 cm) row spacing gave the highest HI (Tables 1 and 2).

Discussion

In linseed, the yield components vary greatly with differences in local environmental conditions, variety and crop density. In the present study the number of capsules per plant was observed to vary as a function of crop density and available space. The higher number of capsules per plant obtained at low density resulted from the production of more secondary (panicle) branches and a greater number of capsules per panicle branch. It was observed during the course of experiments that a smaller number of flowers emerging at the end of the panicle branches set capsules compared with flowers formed earlier, and those capsules that did develop had a smaller number of seeds. The higher number of capsules per plant at low densities, which were unaffected by row spacing, is consistent with the work of Albrechtsen and Dybing (1973), Gubbels (1978) and Freer (1992). The variation in the two years could be the effect of climatic conditions. It was moist and relatively cool during 1993, while it was hot and dry in 1994. Diepenbrock and Porksen (1993) reported that the number of capsules per plant was mainly determined genetically and by density and availability of space. Chandra and Makhija (1979) measured a range of 7 to 65.5 capsules per plant.

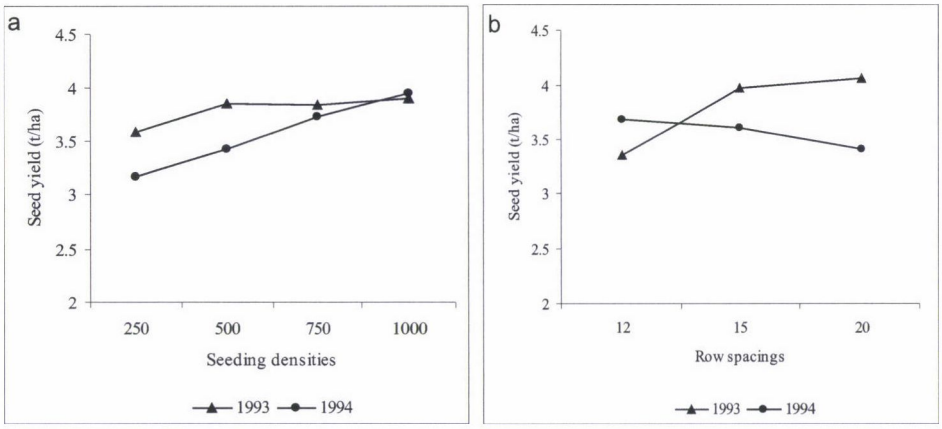

Fig. 4. Effects of (a) seeding density and (b) row spacing on seed yield (t/ha)

The maximum number of seeds per capsule recorded in the two years was 7.6–7.7. However, it was generally observed that the capsules did not produce a uniform number of seeds. Albrechtsen and Dybing (1973) reported that environmental factors such as moisture, temperature and sunlight influenced the total number of seeds. The earlier maturity in dense stands may not allow the maximum number of seeds to set. In both years of the study, the seeds per capsule was found to be higher at low seed density. The slow maturity of well-established plants at lower densities would have encouraged a greater number of seeds per capsule. The greater number of seeds at lower densities is consistent with the results of Freer (1992). A small but consistent decrease in the seeds per capsule with an increase in seed density showed that, to a certain extent, this is a density-responsive variable. Turner (1987) also observed a higher number of seeds per capsule at low density.

Individual seed weight is a component of yield, but remains relatively stable and is, to a great extent, determined genetically. Individual or thousand-seed weight varies according to the cultivars. However, slow maturity at lower densities may have resulted in the greater translocation of photosynthates from lower plant parts to maturing capsules, thereby increasing TSW. Freer (1992) observed higher TSW at lower sowing densities.

Seed yield is the product of the various yield components (number of plants per unit area, number of capsules per plant, number of seeds per capsule and TSW). Although the latter three components all decreased with increasing density, seed yield per unit area progressively increased as the sowing density increased, suggesting that plant population is the dominant component. However, the non-significant differences between the mid-range densities suggests that the yield components (capsules, seeds and TSW) could compensate for small differences in plant density, but not for wider differences. The results of these experiments suggested that seeding densities had no significant effect on

seed yield, except that a very low seeding density might reduce the final yield to some extent. Bilgili et al. (2003) also reported similar results for forage turnip. On the other hand, higher seed density would increase inputs and the risk of lodging under unfavourable conditions. The mid-range densities appear to be optimum. The yield differences in the two years may have been the result of the environmental conditions prevailing during the crop season. The moist, cool growing season of 1993 extended the total length of the crop life cycle, which would have affected total yield, while the hot dry growing season of 1994 hastened maturity. The difference of 11 days from sowing to harvesting could also be responsible for this difference in yield. A significant linear relationship between crop life cycle and total dry matter production has been reported by Hassan and Leitch (2001). In a commercial situation it would be desirable to select a sowing density based on knowledge of local weather conditions. If there were a risk of heavy rains and storms during the maturity stage of the crop, a lower density would be more profitable. In low risk lodging situations higher density might yield more. According to Gubbels (1978), lodging seriously affects yield. Plants at lower plant densities with correspondingly higher rates of basal branches, lodged less than those at higher plant densities. The results from this experiment are consistent with the above findings. On the other hand, Turner (1987) and Freer and Sansome (1991) observed a reduction in seed yield with an increase in seed density.

Low seed density promoted reproductive biomass. The higher value of CI at low seed densities indicated that the distribution of dry matter shifted towards reproductive biomass. The difference between the two experimental years suggests that the weather prevailing during the crop season is the most important factor. Higher ratios of CI at lower density were reported by Diepenbrock and Porksen (1992). Seed density and row spacing had an inconsistent effect on the ratio of seed yield to biomass (harvest index, HI). These findings are in contrast to those of Diepenbrock and Porksen (1992), who recorded 46.96 and 22.63% HI from plant densities of 200 and 1200/m^2, respectively. However, this could be related to the total rainfall in the two years and consequently the total dry matter production. A total of 391 and 229 mm of rainfall was received during 1993 and 1994, respectively.

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Acta Agronomica Hungarica, 53(3), pp. 319–327 (2005)

EFFECT OF LEAD STRESS ON THE GROWTH AND METABOLISM OF *ERUCA SATIVA* M. SEEDLINGS

F. A. FAHEED

BOTANY DEPARTMENT, FACULTY OF SCIENCE, SOUTH VALLEY UNIVERSITY, SOHAG, EGYPT

Received: 2 July, 2004; accepted: 19 August, 2005

Among the toxic elements, lead has great importance because of its harmful effects on animal and human health. Heavy metals are known as growth inhibitors and consequently exhibit toxicity symptoms in plants. The effect of different concentrations of lead on seed germination, seedling growth and some metabolites of *Eruca sativa* plants was examined. The percentage of seed germination gradually decreased with an increase in the lead concentration. A considerable reduction in fresh and dry matter as well as shoot and root length was obtained as a result of increasing lead concentration. Among these parameters, total root length was a more sensitive parameter than shoot length at every lead concentration. The proline content increased, in most cases, with increasing lead concentration. The same trend was observed for the free amino acid content. Soluble protein generally increased with an increase in lead concentration. In contrast, the total protein gradually decreased as the lead concentration increased, particularly at the highest levels used.

Key words: amino acid, proline, lead, stress, growth, *Eruca sativa*

Introduction

Metals occur naturally, and several of them are essential components of global ecosystems. Some metals, such as copper (Cu) and zinc (Zn), are essential to life, whereas others, such as lead (Pb) and mercury (Hg), are not known to perform a useful biochemical function (Allan, 1997). Environmental pollution by metals became extensive when mining and industrial activities increased in the late 19th century.

Soil pollution by toxic metals is one of the most serious problems facing the environment. Plants have frequently been used as indicators to detect metal pollution (Baker et al., 1994). Toxic heavy metals may be transmitted in the food chain and because of their high toxicity they cause a threat to crop production and to animal and human health (Korentejar, 1991). However, heavy metals may be introduced into the soil through the application of fungicides, fertilizers and sewage sludge (Levi-Minzi and Riffaldi, 1978). It has been shown that heavy metal stress affects many physiological and biochemical processes in plants, resulting in the alteration of metabolic pathways (Van Assche and Clijstres, 1990). Lead is one of the most dangerous heavy metals, which can be accumulated in soil and therefore taken up by plants, inducing abiotic stress (Huang and Cunningham, 1996). Also, plants exposed to high levels of lead have been reported to exhibit reduced rates of photosynthesis, growth inhibition

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and the browning of root tips (Kahle, 1993), a decrease in water and nutrient uptake, and finally death (Sanita di Toppi and Gabbrielli, 1999). One mechanism by which many plants and algae respond to and apparently detoxify toxic heavy metals is the production of proline (Delauney and Verma, 1993; Schat et al., 1997; Shah and Dubey, 1998; Mehta and Gaur, 1999; Verma, 1999). Siripornadulsil et al. (2002) showed that proline plays an important role in ameliorating environmental stress, including heavy metal stress, in plants and microorganisms. The accumulation of amino acids and proline in stressed plants is also associated with reduced damage to membranes and proteins (Alia et al., 1997; Shah and Dubey, 1998; Verma, 1999).

Recently, remediation technologies have been elaborated for treating metal-contaminated soils. These include both ecological and engineering technologies (Impens et al., 1991; Logan, 1992). However, toxicity is influenced by a large number of soil and botanical characteristics. Kádár (1995) considered that the heavy metal uptake of plants depended on local (site) characteristics such as soil type, farming patterns, climate, etc.

The aim of the present work was to obtain factual results on the lead uptake of *Eruca sativa* seedlings after exposing the seeds to different lead concentrations and on its effects on growth parameters and physiological responses.

Materials and methods

Plant material

Samples of *Eruca sativa* M. were collected from two agricultural areas differing in their contamination with lead, as shown in previous studies (Table 1). These samples were washed in tap water and separated into roots and shoots. The plant materials were then dried in an aerated oven at 75°C until constant mass to determine root and shoot dry weight. Five replicates were used.

Effect of heavy metal stress on seed germination and seedling growth of Eruca sativa *plants*

The seeds were surface sterilized with 1% (m/v) $HgCl_2$ for 5 min and then washed thoroughly with distilled water before germination. A group of 50 seeds were floated in each Petri dish containing 15 ml of distilled water supplemented with different concentrations of lead in the form of lead acetate (control, 0.1, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mM of lead) and incubated at 27°C in the dark for one week. At the end of the experiment total fresh weight, and the length of shoots and roots per plant were determined. The percentage of seed germination was also estimated. The seedlings were washed twice with distilled water, oven-dried at 75°C for 24 h to obtain the dry weight and then ground to a fine powder. Three replicates were used.

Determination of Pb in soil and plant material

Analytical determinations were carried out according to the method recommended by the Community Bureau of Reference (CBR; Quevauviller et al., 1993). Digestion with concentrated nitric acid was conducted in a closed Teflon PFA vessel at 120°C. The lead concentration in plant and soil samples was determined using an atomic absorption spectrometer (Perkin-Elmer MVD) with an acetylene-air flame.

Table 1
Lead concentrations (mg/g dry weight) of test samples from different agricultural areas

Pb concentration	A	B
Soil	0.378	0.450
Shoot	0.010*	0.021**
Root	0.007*	0.190*
$LSD_{5\%}$	0.025	0.163
$LSD_{1\%}$	0.042	0.269

A: Land far from contamination; B: Contaminated land; *,** Significant at the 0.05 and 0.01 probability levels, respectively

Determination of total and soluble protein, free amino acid and free proline

To estimate the soluble proteins, powdered tissue samples were boiled in distilled water for two hours. After cooling, the water extract was centrifuged and the supernatant was decanted and made up to a known volume with distilled water. The insoluble protein residue remaining after extraction of the water-soluble fraction was treated with NaOH. Soluble and insoluble proteins were determined according to Bradford (1976) using bovine serum albumin as standard.

Total free amino acids were analysed according to Moore and Stein (1948) using L-lysine as a standard. Free proline was extracted and determined according to Bates et al. (1973) using L-proline as a standard.

The results were statistically analysed using Student's t-test to compare the means of the treatments at the 5% and 1% significance levels.

Results and discussion

The results in Table 1 show that the lead concentration in plant material and soil obtained from an agricultural site described as highly contaminated, due to its proximity to main roads, was significantly higher than that of the other site (far from contamination). Since the production of *E. sativa* on a small scale is sufficient for human needs, the plants are cultivated near roads or in house gardens. This land contains a higher concentration of lead emitted by vehicles using petrol as fuel. This explains the differences in lead uptake by plants at the two sites, as was previously reported (Huang and Cunningham, 1996).

When the seeds of *E. sativa* plants were exposed to different concentrations of lead (0.1–3.5 mM), its concentration in plant tissues showed a marked increase, particularly at the highest levels (2.5, 3.0 and 3.5), compared with the control (Fig. 1). Increasing metal concentration was found to have an important effect on accelerating the development of phytotoxicity symptoms (Máthé-Gáspár and Anton, 2002).

The results shown in Figure 2 indicated that increasing lead concentration reduced the seed germination percentage. This was particularly pronounced at the highest concentrations used.

The reduction observed for all measured growth parameters was highly significant, especially when relatively high concentrations were used (Figs. 3 and 4). Among these parameters, the total root length was more sensitive than the shoot length at all the lead concentrations used, as was previously reported (Baker and Walker, 1989; Kahle, 1993; Takács et al., 2001; Nadia et al., 2002).

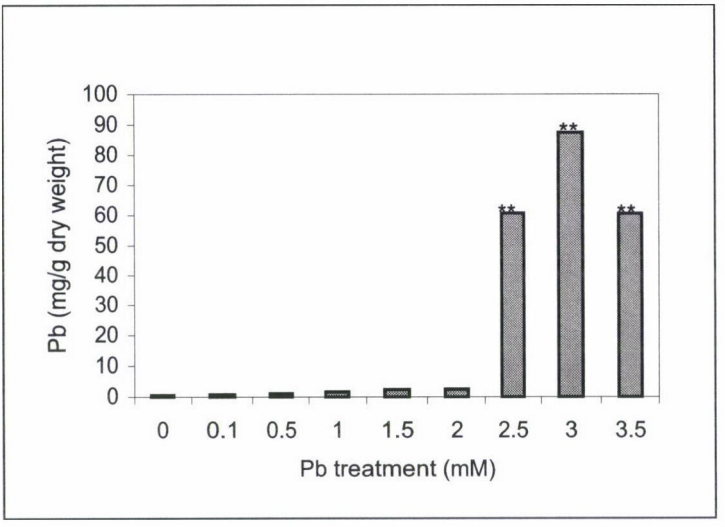

Fig. 1. Lead accumulation in whole plants of *E. sativa*
** Significant at the 0.01 level of probability

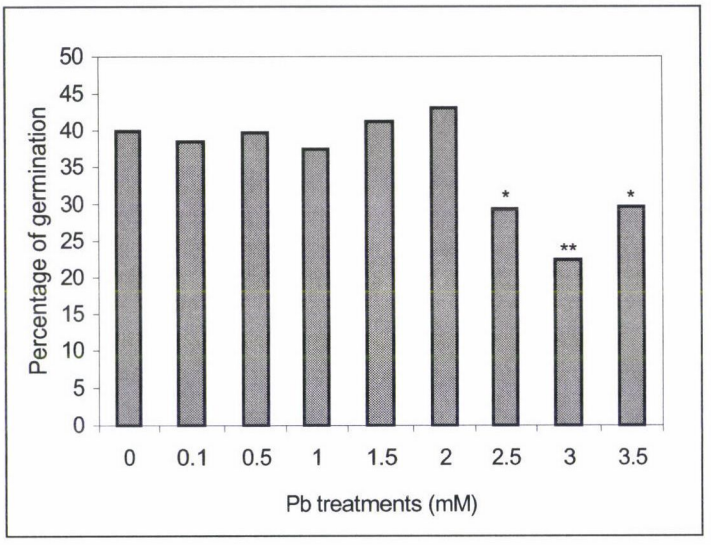

Fig. 2. Effect of different lead concentrations on seed germination of *E. sativa* plants
*,** Significant at the 0.05 and 0.01 probability levels, respectively

The results in Figure 5 and Table 2 show that as the lead concentration increased the proline content also increased, reaching the highest value at 2.5 mM, then slightly decreasing at the two highest concentrations used. These results suggest that proline reduces heavy metal stress by the detoxification of the free radicals produced as a result of lead poisoning, and that proline may physically quench singlet oxygen or react directly with hydroxyl radicals (Alia et al., 1997; 2001; Siripornadulsil et al., 2002).

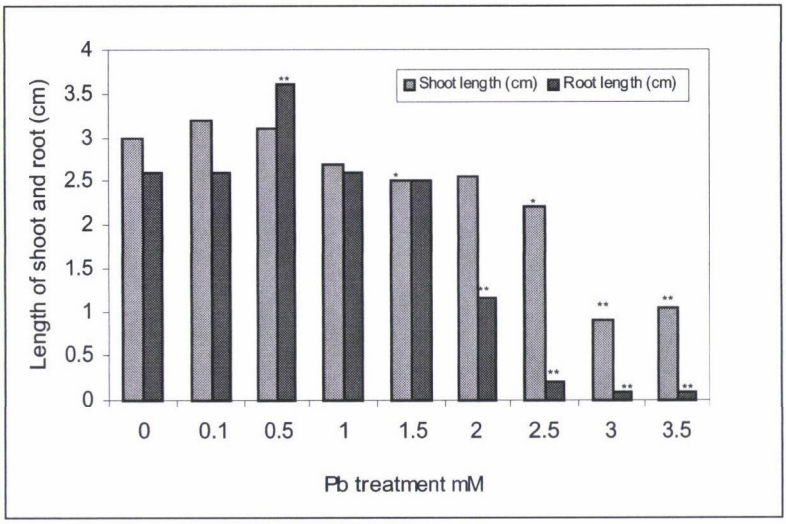

Fig. 3. Effect of different lead concentrations on shoot and root length (cm) of *E. sativa* plants
*,** Significant at the 0.05 and 0.01 probability levels, respectively

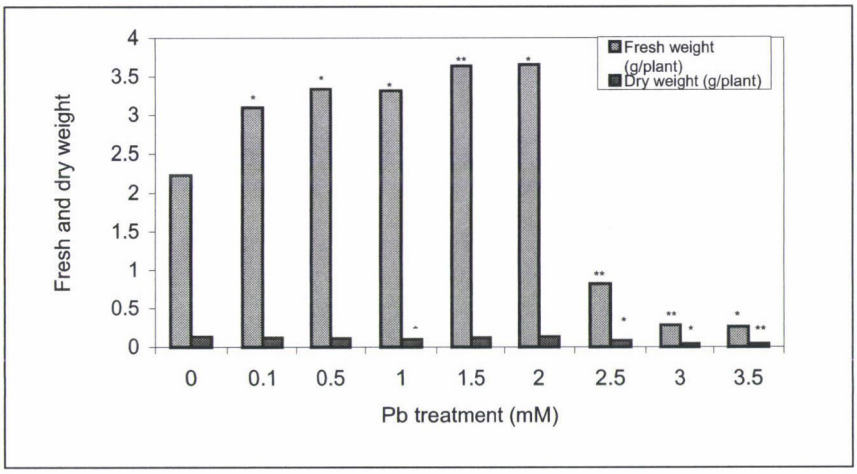

Fig. 4. Effect of different lead concentrations on the fresh and dry weight (g/plant) of *E. sativa* plants
*,** Significant at the 0.05 and 0.01 probability levels, respectively

Table 2
Proline content (mg/g dry weight) of test samples from different agricultural areas

Proline content	A	B
Shoot	0.254	0.712
Root	0.286	1.818**
$LSD_{5\%}$	1.039	0.076
$LSD_{1\%}$	2.397	0.176

A: Land far from contamination; B: Contaminated land.**: Significant at 0.01 probability level

It is well known that heavy metals induce water deficit conditions, and this effect increased with an increase in the lead concentration. At high lead concentrations (3.0 and 3.5 mM) the synthesis of proline was reduced. This was concomitant with a reduction in seed germination and seedling growth, indicating that the proline accumulated at relatively low concentrations of lead (0.1–2.0 mM) may play an important role as an osmoprotectant, as was previously reported (Schat et al., 1997).

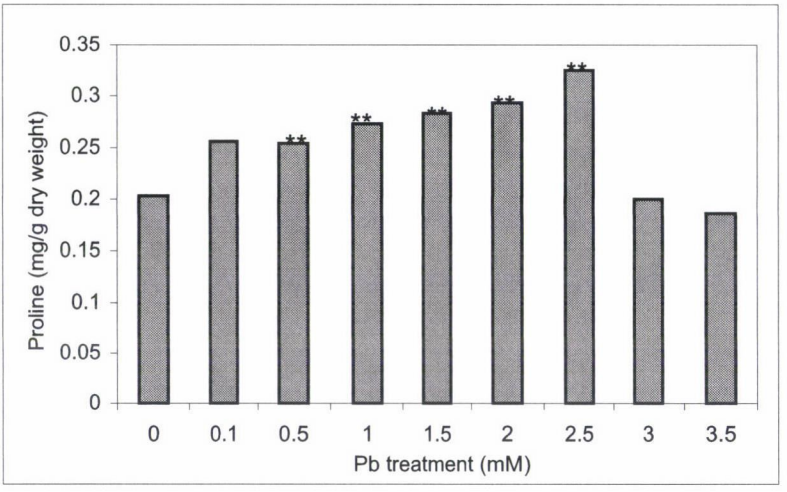

Fig. 5. Effect of different lead concentrations on the proline content (mg/g dry weight) of *E. sativa* plants
** Significant at the 0.01 probability level

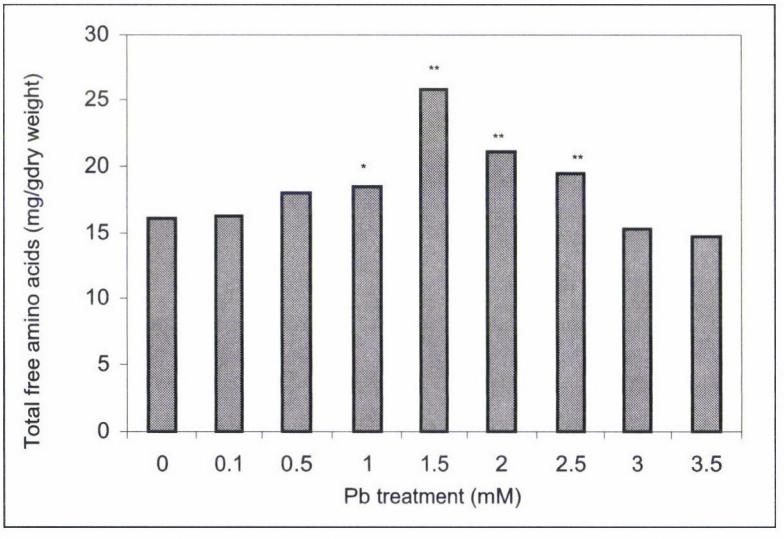

Fig. 6. Effect of different lead concentrations on the content of total free amino acids (mg/g dry weight) of *E. sativa* plants.*,** Significant at the 0.05 and 0.01 probability levels, respectively

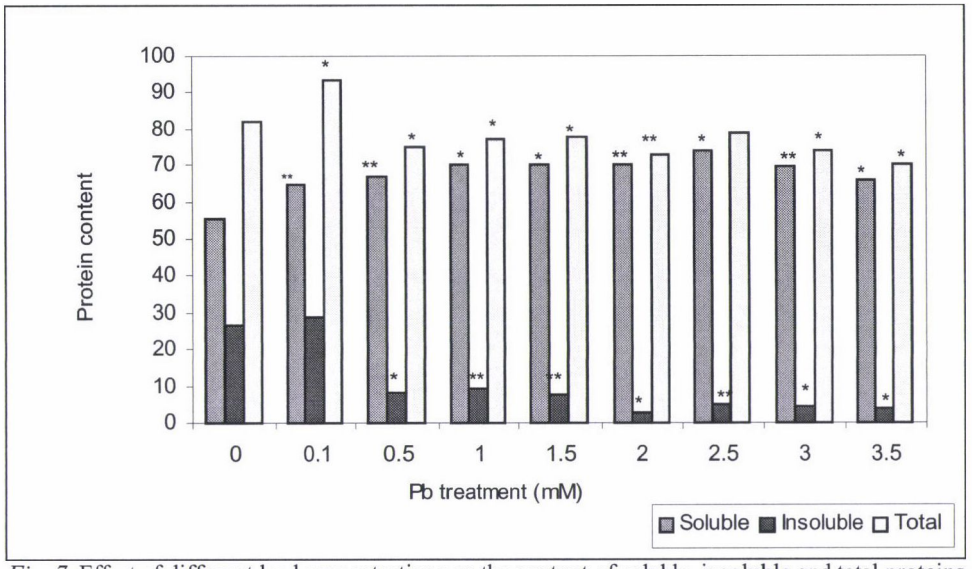

Fig. 7. Effect of different lead concentrations on the content of soluble, insoluble and total proteins (mg/g dry weight) of *E. sativa* plants

*,** Significant at the 0.05 and 0.01 probability levels, respectively

Proline is considered to be involved in osmotic adjustment, acting as a compatible osmolyte and functioning as a protector of macromolecules such as proteins and membranes and as a nitrogen storage compound and energy source after stress (Aspinall and Palag, 1981; Delauney and Verma, 1993).

In *E. sativa* seedlings the total free amino acid content only increased up to a lead concentration of 1.5 mM compared to the control samples (Fig. 6). This result is in good agreement with results obtained previously (Nover et al., 1989).

Soluble and total protein first increased then remained constant as the lead concentration increased, particularly at the three highest levels used, compared with the control (Fig. 7). In contrast, the insoluble protein gradually decreased compared with the control as the lead concentration increased (Fig. 7). The results of Singh et al. (1997) suggest that the total nitrogen in the roots and shoots of different mungbean cultivars generally increased after treatment with lead acetate. In addition, Sinha et al. (1988) found a slight increase in the protein and nitrogen content in pea due to lead pollution. An increase in soluble protein and organic nitrogen contents also occurred in sesame and mungbean roots and shoots in the presence of lead during early growth stages (Kumar et al., 1993; Bharti and Singh, 1993; Singh et al., 1994).

Conclusions

The data in this work indicate the presence of a positive correlation between the lead concentration of cultivated land and the accumulation of lead in cultivated plants. Therefore, the cultivation of *E. sativa* near roads with heavy traffic is not recommended.

On the basis of this data there is a fundamental difference in the growth parameters and metabolic changes, reflected in a decrease in germination, fresh and dry mass, shoot and root length, total protein content and radical changes in the total free amino acids. The only parameter indicating the toxicity of Pb at high concentrations was the increase in proline content.

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Acta Agronomica Hungarica, 53(3), pp. 329–337 (2005)

YIELD, YIELD COMPONENTS AND PHYSICO-CHEMICAL CHARACTERS OF THE RATOON CROP OF IRANIAN RICE CULTIVARS

N. B. JELODAR

DEPARTMENT OF PLANT BREEDING, COLLEGE OF AGRICULTURE, UNIVERSITY OF MAZANDARAN, SARI, MAZANDARAN, IRAN

Received: 8 March, 2004; accepted: 5 May, 2005

The ratooning ability of five Iranian rice cultivars and the grain quality of the ratoon crop were evaluated in a randomized complete block design with four replications in consecutive years from 1999 to 2001 to select promising lines for economical ratoon culture. The main crop was harvested by cutting 30 cm above the soil surface for regrowth of the second (ratoon) crop. The ratoon grain yields (kg/ha) varied greatly among the cultivars, ranging from 144.9 (Haseni) to 1472.1 (Sang-e-Tarom). In general, the ratoon crops had fewer fertile spikelets, fewer spikelets per panicle, shorter panicle length and fewer primary and secondary branches per panicle than the main crop. The gelatinization temperature and gel consistency of rice grains from ratoon crops were lower than those of the main crops. The amylose content (%) of the grains from ratoon crops varied from 23.1 (Sang-e-Tarom) to 21.4 (Dailamani).

Key words: yield, yield components, ratoon, Iranian rice, gelatinization temperature, amylose

Introduction

Ratooning is the practice of allowing the crop to regenerate after cutting in order to harvest a second crop. In areas where adequate water is available after the main season, rice ratooning could be practiced as an alternative to double-cropping. Ratooning is basically a varietal character and it differs among cultivars (Chatterjee et al., 1982). Its benefit is that it does not involve costs and labour in raising seedlings in a nursery or in preparatory cultivation of the land. Increasing the ratooning ability is thus an important breeding objective. Ratooning ability can be expressed as the ratio of ratoon tillers to the main crop tillers (Chauhan et al., 1989). The first rice cultivar developed for ratooning at Nagenahalli, India was S-684, with the ratoon crop maturing in 35% the time needed to grow the main crop (Krishnamurthy, 1988).

The best time to harvest the main crop for good ratooning is when the culms are still green (Grist, 1965). The amount of carbohydrate reserves in the culm at maturity generally has a positive but weak correlation with the ratoon crop yield (Bollich et al., 1988). Many results suggest that Japonica varieties have better ratooning ability than Indica varieties (Krishnamurthy, 1988). Intermediate to late maturing varieties usually produce higher ratoon yields than early maturing ones (Krishnamurthy, 1988). The data available on ratoon rice indicates that yields of up to 50% of the main crop are possible (Krishnamurthy, 1988).

0238–0161/$ 20.00

In addition to shorter growth duration and lower production costs, ratoon crops require less irrigation water than main crops (Bollich et al., 1988). When the ratooning ability of 57 hybrids and 5 varieties was evaluated at IRRI, the ratoon crop yield ranged from 0.1 to 1.81 t/ha, which was up to 70% of the main crop yield (Prakash and Prakash, 1988). Average ratoon yields in research plots have remained relatively constant for 30 years (Bollich et al., 1988).

Rice ratoon cropping systems have not been developed in Iran on a commercial scale mainly because of the lack of suitable cultivars. Despite the availability of data on the ratooning ability of several cultivars, information on their cooking quality, milling quality and nutritive value have not been determined (Krishnamurthy, 1988). Hence, in this research the ratooning ability, milling quality and physico-chemical characteristics of five rice varieties were measured to select promising ones for economical ratoon culture.

Materials and methods

The field experiment was carried out during 1999 to 2001 at Amol Rice Research Station (Mazandaran Province, North Iran). Five early maturity rice cultivars (cvs. Dailamani, Haseni, Nok-Siah, Daei-Shastak and Sang-e-Tarom) were evaluated for the growth parameters, yield and quality of the ratoon crop. Seedlings were transplanted 30 days after seeding at a spacing of 20×20 cm using five seedlings/hill in a randomized complete block design with four replications. The soil was sandy clay with a pH of 6.9. The main crop was fertilized with 50 kg N and 50 kg P_2O_5/ha. After maturity, the plants were cut 30 cm above the soil level. The ratoon crop was not fertilized after the main crop. Sufficient soil moisture was maintained to the end of tillering, then the plots were irrigated until the grain of the ratoon crop matured. Plant regeneration was recorded up to 8 weeks after cutting. Plants with more than one ratoon tiller were included for recording the percentage of regeneration. Ratooning ability was estimated as the ratio of productive tillers in the ratoon crop and main crop (Chauhan et al., 1989). The ratoon yield and its components were also recorded.

Grains of the varieties were collected, cleaned and corrected for moisture. Weighed samples (100 g each) of clean paddy rice were dehusked in triplicate in a laboratory (Amol Rice Research Station) using a Satake Rice Sheller (Japan) equipped with a rubber roller. The distance between the rollers was adjusted depending upon the shape and dimensions of the grains. The brown rice obtained was polished in a McGill Miller No. 2 (Rapsco, Brokshire, TX, USA) to obtain a uniform 6% degree of polish in all samples. Head rice and broken rice grains were separated using a laboratory rice sizing device (Burrows Equipment Co., Evanston, IL, USA) and their percentage rates determined.

The amylose content (%) was estimated by the method of Juliano (1971). The analysis was repeated three times. The gelatinization temperature was estimated from the extent of spreading and clearing of milled rice incubated with 1.7% potassium hydroxide for 23 h at 30°C, using the procedure of Little et al. (1958). Gel consistency determinations were made in duplicate using the procedure of Cagampang et al. (1973). This test is based on the consistency of cold 5.0% milled rice paste in 0.2 N KOH. The consistency was measured as the length of the cold gel in a horizontally-held test tube. The data were statistically analysed using the SPSS package and Student's t-test (Steel and Torrie, 1960).

Results

Among the varieties tested, Sang-e-Tarom produced the largest number of productive tillers per hill (10.4) as a ratoon crop. There were significant differences in the mean length and width of the flag-leaf between the ratoon plants. The ratoon crops of Daei-Shastak and Haseni had a significantly narrower and shorter flag leaf (Table 1).

Comparisons were made between the panicle characters of the ratoon crop in different cultivars, i.e. panicle length, number of primary branches/panicle, number of secondary branches/panicle and spikelets per panicle (Table 1). Panicle length was significantly different. The ratoon crop of Dailamani had the longest panicles and Daei-Shastak the shortest. The number of primary and secondary branches/panicle were also significantly different, the largest number being recorded for Sang-e-Tarom and the lowest for Haseni. In the case of the number of spikelets/panicle Sang-e-Tarom had significantly more spikelets/panicle and Dailamani had significantly fewer.

The results presented in Table 1 show that the largest number of filled grains/panicle in the ratoon crop occurred in Sang-e-Tarom (36.3) and the smallest in Dailamani (12.8). Of all the varieties, Sang-e-Tarom recorded the highest ratoon crop yield (1472.1 kg/ha), which was 22% of its main crop yield (Table 1). The mean ratooning ability varied from 0.72 (Dailamani) to 0.43 (Haseni) (Table 2).

The results obtained for total milled rice are given in Table 3. The percentage of total milled rice decreased significantly in the ratoon crop of Dailamani and Nok-Siah. The total milled rice of the ratoon crop of Haseni, Daei-Shastak and Sang-e-Tarom was found to be non-significant compared to the total milled rice of the main crop (Table 3). The percentage of total milled rice of the ratoon crop, however, varied between the cultivars, Sang-e-Tarom showing the highest value (67.9%) and Nok-Siah the lowest (62.7%). The broken rice percentage in the ratoon crop of Haseni and Daei-Shastak was not significantly different from that of the main crop.

The percentage hulls in the ratoon crop of Dailamni and Daei-Shastak did not differ significantly compared to that of the main crop. There were significant differences between the rice bran values of the ratoon crop and the main crop except for Daei-Shastak, the values being significantly higher in the ratoon crop for Dailamani, Haseni and Nok-Siah.

The percentage of head rice in the ratoon crop of Dailamani, Nok-Siah and Sang-e-Tarom was significantly different compared to that of the main crop, while that of Haseni and Daei-Shastak was non-significant.

There were significant differences between the amylose contents of grains from the ratoon and main crops in Dailamani and Nok-Siah (Table 4). The highest amylose content was recorded for the main crop of Sang-e-Tarom and the lowest for that of Haseni. The amylose contents of the ratoon crop in Haseni, Daei-Shastak and Sang-e-Tarom were not significantly different from those of the main crop (Table 4).

Table 1

Varietal differences in yield components of ratoon crops

Varieties	No. of productive tillers/hill	Length of flag leaf (cm)	Width of flag leaf (mm)	Panicle length (cm)	No. of primary branches/panicle	No. of secondary branches/panicle	No. of spikelets /panicle	No. of filled grains/panicle	No. of unfilled grains/panicle
Dailamani	9.0 ± 1.4 b	24.6 ± 2.0 a	9.0 ± 0.2 ab	18.4 ± 3.0 a	3.3 ± 0.5 c	6.8 ± 0.5 b	30.2 ± 1.8 d	12.8 ± 0.9 c	18.33 ± 2.35d
	(12.5 ± 2.2)	(24.3 ± 1.6)	(5.7 ± 1.2)	(24.3 ± 1.6)	(5.7 ± 1.2)	(10.9 ± 3.6)	(118.2 ± 12.5)	(112.2 ± 13.1)	(6.0 ± 0.9)
Haseni	6.8 ±2.4 c	15.9 ± 1.5 c	7.0 ± 0.2 c	15.1 ± 1.6 c	3.2 ± 0.5 c	4.1 ± 0.3 c	32.7 ± 4.2 d	8.6 ± 0.6 d (	23.77 ± 6.52c
	(15.7 ± 1.8)	(25.1 ± 1.4)	(13 ± 0.3)	(25.6 ± 3.3)	(5.9 ± 1.1)	(11.3 ± 3.4)	(107.4 ± 7.6)	102.4 ± 7.4)	(5.0 ± 1.1)
Nok-Siah	7.2 ± 1.7 c	17.7 ± 2.1 b	10 ± 0.1 a	14.3 ± 1.5 ce	3.4 ± 0.7 bc	6.8 ± 0.5 b	39.1 ± 4.2 c	12.9 ± 5.2 c	26.3 ± 9.71b
	(14.2 ± 1.2)	(22.6 ± 1.2)	(14 ± 0.2)	(26.4 ± 1.7)	(5.7 ± 1.6)	(11.3 ± 4.1)	(129.1 ± 11.1)	(119.8 ± 16.7)	(11.2 ± 1.2)
Daei-Shastak	9.8 ± 1.3 ab	15.3 ± 3.1 c	7 ± 0.1 c	14.6 ± 0.7 c	3.6 ± 0.9 b	6.9 ± 1.0 b	45.1 ± 4.8 b	15.6 ± 3.8 b	30.71 ± 4.61a
	(16.1 ± 1.8)	(22.6 ± 1.2)	(11.0 ± 0.2)	(22.5 ± 1.9)	(4.7 ± 1.1)	(10.3 ± 3.9)	(101.2 ± 18.7)	(94.0 ± 20.8)	(7.0 ± 1.3)
Sang-e-Tarom	10.4 ± 0.8 a	17.9 ± 11.9 b	8.0 ± 0.2 b	16.5 ± 1.6 b	3.9 ± 0.9 a	7.9 ± 1.2 a	61.0 ± 5.2 a	36.3 ± 5.2 a	24.82 ± 5.19c
	(17.7 ± 2.2)	(22.8 ± 2.0)	(12.0 ± 0.3)	(24.7 ± 2.6)	(5.7 ± 1.2)	(14.0 ± 4.2)	(124.2 ± 3.2)	(120.2 ± 16.4)	(3.8 ± 0.8)
F-value									
Year (YR)	66.04**	1500.79**	43.58**	314.8**	267.78**	1.15^{ns}	205.45**	7979.39**	13857.65**
Cultivar (CV)	1006.06**	2664.13**	0.28^{ns}	168.15**	782.56**	0.85^{ns}	15.67**	1050.04**	2229.77**
YR × CV	45.54**	335.55**	2.02^{ns}	422.65**	281.29**	1.03^{ns}	14.09**	1551.71**	770.08**

**: Significant at the 1% level; main crop values are given in parentheses; any two means in the same column followed by the same letter are not significantly different at the 5% level; ns: non-significant

Table 2

Varietal differences in the yield of the ratoon crop, the main crop/ratoon crop yield ratio and ratooning ability

Varieties	Yield						Ratooning ability
	1999		2000		2001		
	Rc (Mean ± SE)	Rc/Mc (%)	Rc (Mean ± SE)	Rc/Mc (%)	Rc (Mean ± SE)	Rc/Mc (%)	
Dailamani	583.8 ± 91.7 c (4463.5 ± 490.7)	13	420.3 ± 168.7 c (3673.3 ± 384.6)	11	601.7 ± 120.1 b (4773.2 ± 285.2)	14	0.72
Haseni	144.9 ± 45.2 e (3992.5 ± 164.5)	4	284.1 ± 92.4 d (4104.3 ± 86.6)	7	391.2 ± 68.7 c (4051.2 ± 201.7)	10	0.43
Nok-Siah	433.4 ± 71.2 d (4483.5 ± 361.3)	10	456.5 ± 109.5 c (4918.6 ± 492.0)	9	438.4 ± 211.5 c (4371.2 ± 472.1)	10	0.51
Daei-Shastak	694.3 ± 81.2 b (4493.0 ± 524.7)	20	528.4 ± 35.4 b (3177.3 ± 641.9)	17	578.5 ± 209.7 b (3978.2 ± 291.2)	14	0.61
Sang-e-Tarom	1014.4 ± 130.7 a (4727.5 ± 384.8)	22	928.4 ± 35.4 a (4169.0 ± 373.5)	22	1472.1 ± 408.3 a (4561.0 ± 307.9)	32	0.59

SE: Standard error; Mc: Main crop; Rc: Ratoon crop; main crop values are given in parentheses; any two means in the same column followed by the same letter are not significantly different at the 5% level.

Table 3

Varietal differences (Mean ± SE) in milling characters (%) of main and ratoon crops

Varieties	Main crops					Ratoon crops				
	Total milled rice	Hulls	Rice bran	Head rice	Broken rice	Total milled rice	Hulls	Rice bran	Head rice	Broken rice
Dailamani	69.2 ± 3.2	21.5 ± 1.1	9.3 ± 0.6	53.4 ± 1.7	25.1 ±1.0	64.2 ± 2.9**	22.6 ± 1.2^{ns}	11.3 ± 0.7**	44.3 ±1.9**	10.2 ± 0.8**
Haseni	65.2 ± 3.0	24.6 ± 1.1	10.2 ± 0.7	43.8 ± 1.8	22.8 ± 1.1	64.6 ± 3.7^{ns}	21.9 ± 1.7**	12.4 ± 1.3**	42.0 ± 2.1ns	21.6 ± 1.2^{ns}
Nok-Siah	69.0 ± 2.9	22.8 ± 1.0	8.2 ± 0.8	61.4 ± 1.9	18.1 ± 0.9	62.7 ± 3.2**	27.1 ± 1.1**	10.2 ± 0.6**	39.7 ± 1.9**	23.0 ± 1.1**
Daei-Shastak	65.8 ± 2.7	25.4 ± 0.6	8.8 ± 0.8	57.5 ± 1.8	8.6 ± 0.6	65.1 ± 3.1^{ns}	25.9 ± 1.2^{ns}	9.0 ± 0.8^{ns}	56.8 ± 2.1^{ns}	8.3 ± 1.2^{ns}
Sang-e-Tarom	68.5 ± 2.2	21.6 ± 0.9	8.4 ± 0.6	59.8 ± 1.8	24.7 ± 0.9	67.9 ± 3.0^{ns}	24.8 ± 1.1**	7.3 ± 0.7*	50.3 ± 2.2**	8.8 ± 0.9**

**: Significant at the 1% level; *: Significant at the 5% level; ns: Non-significant; SE: Standard error

Table 4
Varietal differences (Mean ± SE) in three physico-chemical characters of main and ratoon crops

Varieties	Amylose (%)	Gel consistency (mm)	Gelatinisation temperature (°C)
		Main crop	
Dailamani	24.1 ± 1.2	45.2 ± 2.1	4.3 ± 0.1
Haseni	22.4 ± 0.9	40.5 ± 1.6	6.6 ± 0.3
Nok-Siah	24.2 ± 1.2	39.9 ± 1.4	4.5 ± 0.2
Daei-Shastak	22.6 ± 0.8	54.7 ± 1.9	4.4 ± 0.1
Sang-e-Tarom	24.4 ± 1.1	56.5 ± 2.1	3.9 ± 0.1
		Ratoon crop	
Dailamani	21.4 ± 0.9**	29.5 ± 1.1**	3.3 ± 0.2**
Haseni	21.5 ± 1.3^{ns}	29.5 ± 1.4**	5.6 ± 0.4**
Nok-Siah	22.7 ± 0.9**	27.0 ± 1.3**	3.2 ± 0.2**
Daei-Shastak	22.1 ± 1.0^{ns}	42.5 ± 2.1**	3.6 ± 0.3**
Sang-e-Tarom	23.1 ± 0.8^{ns}	32.0 ± 1.1**	3.1 ± 0.1**

**: Significant at the 1% level; ns: Non-significant; SE: Standard error

Physico-chemical analysis revealed that there were significant differences in gel consistency and gelatinization temperature between the main crops and the ratoon crops. The gelatinization temperature of the main crop was higher than that of the ratoon crop (Table 4).

Discussion

Sang-e-Tarom produced the largest number of productive tillers per hill (10.4) as a ratoon crop among all the varieties. Many tillers of the ratoon crop were produced from the base of the plant, and bore long panicles and unfilled grains, because of the prevailing cold temperature, while tillers from the buds of the upper nodes bore very short panicles and filled grains. Similar results were reported by Dishman (1961). Unproductive tillers were produced from the ratoon crops of all varieties, which was not beneficial in increasing the yield.

The results presented in Table 1 show that the largest number of unfilled grains in the ratoon crop occurred in Daei-Shasatak (30.7) and the smallest in Dailamani (18.33). This high number of unfilled grains in the ratoon crop was due to cold temperatures during late September and October, which delayed the regrowth of these varieties and resulted in high sterility. Unfilled grains were more frequent in the panicles of the ratoon crop than in the main crop (Table 1). This suggests that ratooning ability is a varietal character and also influenced by the environment.

A reduction in the number of filled grains per panicle was observed in the ratoon crop of all the cultivars compared to the main crop. The number of filled grains per panicle appeared to be the most responsive yield component. The vegetative growth of the ratoon crop was much shorter than the main crop, reducing the yield of grains in the ratoon crop.

The grain yields varied depending on year and cultivar (Table 2). Of all the varieties, Sang-e-Tarom gave the highest ratoon crop yield (1472.1 kg/ha), which was 22% of its main crop yield. This can be attributed to genetic differences between the varieties, as suggested by Palchamy and Kolandaiswamy (1982). Sang-e-Tarom had much faster, more uniform regrowth, which may explain its superior ratoon yield. Apparently, the main crop and the ratoon crop differ in their responses to environment, possibly because of cultivar differences, but also due to climatic differences during the critical period for the development of the main and ratoon crops (Table 2). Similar results were reported by Turner and Jund (1993).

The percentage of total milled rice in the ratoon crop varied between the cultivars. The milling quality of rice is based primarily on the yield of whole grain rice obtained. Grain breakage during processing is a serious problem in all rice-growing areas. An investigation by Singh et al. (1990) revealed that the flooding of paddy had an adverse effect on the milling quality of rice.

The ratoon crop of Daei-Shastak, which has medium long grains, gave the highest head rice percentage (56.8%) and Nok-Siah the lowest (39.7%). The losses in head rice recovery might be due to a change in relative humidity during the ripening of ratoon rice kernels and the development of fissures (stress cracks) in the kernel due to internal stress caused by uneven moisture content and drying.

There were significant differences between the amylose contents of the ratoon crop grains and the main crop. The differences in amylose content evidently arose due to the differences in the stature of the grains. Many researchers have stated that the temperature during grain ripening affected amylose content (Paule, 1977; Gomez, 1979). Amylose content generally decreases as the mean temperature increases (Table 4).

Physico-chemical analysis revealed that there were significant differences in the gel consistency and gelatinization temperature between the main crops and the ratoon crops. The gelatinization temperature of the main crop was higher than that of the ratoon crop. This agreed with the findings of Khush et al. (1979), Beachell and Stansel (1963) and Blakeney (1996). Khush et al. (1979) stated that environmental conditions, such as the temperature during grain ripening, influenced the gelatinization temperature. A higher ambient temperature during grain development results in starch with a higher gelatinization temperature. Gelatinization temperature is known to vary under different temperature regimes. Beachell and Stansel (1963) pointed out that cooler temperatures, especially during ripening, produced starch with a lower gelatinization temperature. Juliano et al. (1964) concluded that higher amylose content generally resulted in a higher gelatinization temperature. Amylose content, gelatinization temperature and gel consistency are the major determinants of the cooking and eating qualities of milled rice (Juliano et al., 1964; Khush et al., 1979).

In conclusion, yield increases in ratoon rice cultivation might be possible by reducing the negative effects of early autumn cold through the development of cold-tolerant varieties of ratoon rice in Mazandaran Province. The present study emphasizes that, with suitable cultivars and cultural practices, the ratoon cropping of rice in the Mazandaran and Gilan Provinces of North Iran offers an opportunity to effectively utilize land resources which otherwise would not be used between the first crop harvest and the cold season. Thus, ratooning has the potential to significantly increase rice production in this area with minimal additional resources. A rice ratoon crop could be fitted into the overall cropping system to enable two crops to be produced in the period before the cold season. The crop could be advantageously fitted into relay double or triple cropping sequences with other cereals.

Acknowledgements

The technical assistance of E. Hassen-Nattaj and N. Bagheri is gratefully acknowledged. Thanks are due to Professor Chiharu Nakamura for helpful comments on the manuscript. This project was supported by the National Scientific Research Council of Iran and the University of Mazandaran.

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Acta Agronomica Hungarica, 53(3), pp. 339–347 (2005)

DISTRIBUTION OF NUTRIENTS IN THE ACTIVE ROOT AREA OF OLIVE TREES UNDER A DRIP IRRIGATION SYSTEM IN A SANDY SOIL

S. H. A. SHAABAN and M. M. EL-FOULY

FERTILIZATION TECHNOLOGY DEPARTMENT, NATIONAL RESEARCH CENTRE, DOKKI, EGYPT

Received: 24 October, 2004; accepted: 28 June, 2005

A study was carried out on the root distribution of mature olive trees (*Olea europaea,* L., var. *manzanillo*) grown on a sandy soil, irrigated by means of drip irrigation, and on the nutrient distribution in the soil and plants. Soil was sampled at a distance of 20–160 cm from the tree trunks at depths of 0–80 cm. Leaves were also analysed for nutrient content. The roots were concentrated in the area 80–140 cm from the trunk at a soil depth of 20–60 cm, i.e. in the zone with the highest soil water content. In the horizontal layer, magnesium, iron, manganese, zinc and copper mostly decreased at the ends of the wet area, while in the vertical layer, the 0–20 cm surface soil layer was the richest in potassium, calcium, manganese, zinc and copper. Phosphorus concentrations fluctuated. The average values of potassium, magnesium, iron, manganese, zinc and copper concentrations in olive leaves were significantly higher in bearing years than in non-bearing ones. For this reason it is important to increase the doses of fertilizers containing these elements after bearing years to replace the high removal.

Key words: Olea europea L., sandy soil, drip irrigation, nutrient distribution, leaf analysis

Introduction

Egypt has an arid to semi-arid climate. On newly cultivated areas in Egypt fertilizers are applied with the irrigation water (fertigation) to maximize the use of water resources and obtain high fertilizer efficiency. Olive trees (*Olea europaea* L.) occupy a large area of the reclaimed land. Mean yields are considered low to average, one probable reason for which might be an insufficient knowledge of the nutritional status of olive under drip irrigation. The objective of the present work was to investigate the patterns of root and nutrient distribution in the soil and the effect of the fertilization regime on leaf nutrient status under fertigation conditions in sandy soil. This might deliver important information for optimizing fertilizer use through effective fertilizer recommendations.

Materials and methods

Field practices

This study was carried out during four consecutive years (2000–2003) in an olive orchard located near the Cairo-Alexandria desert road to evaluate the nutritional status of olives (*Olea europaea* L.). Soil and leaf samples were collected. Soil samples were taken from equal distances and depths from the trunk of the tree. Soil characteristics were 79% sand, pH 8.92, 7.2% $CaCO_3$

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and 0.37% organic matter. Young trees were planted in 1994 at a density of 400/ha with a base dressing of 31, 8, 86 g N, P_2O_5 and K_2O per tree, respectively. The trees were drip irrigated from underground water containing 275 ppm total soluble solids including 27, 178, 224 ppm SO_4^{2-} Cl^- and HCO_3^-, respectively. Drippers were spaced 25 cm from the tree trunk when planted in 1994 and 100 cm in 2000, with two drippers/tree in the last two years instead of one. Between 1994 and 2002 the fertilizer regimes were determined annually on the basis of soil tests and leaf analysis. Fertilizers were applied through the drip irrigation system. In 2000, 2001 and 2002 the annual rates of application were as follows: 60, 70, 80 g N/tree in equal doses between February and November, 8, 16, 24 g P_2O_5/tree in January, and 144, 168, 192 g K_2O/tree in equal doses between April and December. Micronutrients were applied as a foliar spray containing iron (Fe), manganese (Mn) and zinc (Zn) in compound chelated form four times during the growing period: before flowering, after fruit set, at pit hardening and at the end of the summer. Total doses per year were 320, 215, 110 g/ha for Zn, Mn and Fe, respectively. Urea (1%) was applied to the leaves with the micronutrient mixture. The trees bore fruit in 2000 and 2002, while 2001 and 2003 were non-bearing years.

Experimental

Soil profiles were prepared in the year 2001 by digging a trench 160 cm from the trunk to a depth of 80 cm under three randomly distributed trees. The soil was sampled every 20 cm in both the horizontal and vertical directions. The number of samples was 32 (8×4) for each location. Representative samples were taken from 4-month-old leaves in the spring growth cycle in successive years to cover both productive and non-productive years.

Soil tests and leaf analysis

Leaf samples were collected from adult trees of the Manzanillo variety (dual purpose), which is the prevailing variety in these areas. Leaves were sampled under natural growth conditions from the middle portion of the terminal shoots of the previous season. The lower and apical leaves were discarded (Bouat, 1964). The leaf samples were washed once with tap water and with 0.001 M HCl, then washed with distilled water, after which they were air-dried for 1–2 h, and then dried in a ventilated oven at 70°C for 48 h. The dried samples were ground in a stainless steel mill with a 0.5 mm sieve. The soil samples were analysed for texture with a hydrometer (Kilmer and Alexander, 1949), for pH and EC in a 1:2.5 soil/water suspension (Jackson, 1973), for total $CaCO_3$ with a Collin's calcimeter (Alison and Moodle, 1965) and for organic matter content according to Walkley and Black (1934). The available K, Mg and Ca contents were extracted with NH_4-AOC (Chapman and Pratt, 1978), while P was extracted with $NaHCO_3$ (Olsen et al., 1954). Soil available micronutrients (Fe, Mn, Zn, and Cu) were extracted using DTPA (Lindsay and Norvell, 1978).

The total leaf contents of P, K, Mg, Ca and Na, and of Fe, Mn, Zn and Cu were extracted after dry ashing a sample of 1 g in a muffle furnace at 500°C for 6 h, after which the ash was dissolved in HCl (2N), diluted to 50 ml with distilled water and filtered (Chapman and Pratt, 1978), while total N was determined with the micro-Kjeldahl method (Markaham, 1942), using the boric acid modification described by Ma and Zuazage (1942), and distillation was done using a Buchi 320-N_2 distillation unit. In both the soil and leaf samples K, Ca and Na were measured using a flame photometer (Jenway, PFP 7), while P was measured with a spectrophotometer (Perkin-Elmer, LKB Ultrospec II UV/VIS Spectrophotometer Lambda 2) using the vanado-molybdate colour reaction. Fe, Mn, Zn, Cu and Mg were measured with an atomic absorption spectrophotometer (Perkin Elemer, 1100). The soil data were evaluated using the criteria reported by Ankerman and Large (1974) and Lindsay and Norvell (1978), whereas the leaf analysis data were evaluated according to the criteria suggested by Haselhoff and Blanck (1928), Bouat (1964), Hartmann et al. (1966) and Recalde and Chaves (1975).

Results

Root distribution

Observations on root distribution showed that in 2001 the roots extended to 160 cm from the tree horizontally and 80 cm deep. The areas of greatest root concentration were always found in the superficial soil layer 80–140 cm from the trunk in the upper 20–60 cm layer. More than 70% of the roots were found in these areas. The results indicate that a greater number of roots are found wherever there is a greater quantity of water.

Soil properties

Figure 1 shows that the pH increased in the subsurface soil (40–80 cm) at a horizontal distance of 140–160 cm from the trunk and EC increased at the same distance in the surface soil (0–40 cm), while the $CaCO_3$ and Na contents were higher at 0–40 cm soil depth than at 40–80 cm. Organic matter was also higher at 0–40 cm soil depth than at 40–80 cm and at both depths it decreased with the distance from the trunk. Generally, soil organic matter levels were low.

Nutrients distribution in soil

Figures 2 and 3 show that in a horizontal direction magnesium, iron, manganese, zinc and copper mostly decreased after a distance of 120 cm, whereas calcium increased. In a vertical direction the 0–40 cm soil layer was the richest in potassium, calcium, manganese, zinc and copper, while the phosphorus concentrations fluctuated.

Nutrients in olive leaves

As can be seen from Figure 4, K was deficient in the leaves of non-bearing trees (2001 and 2003). Experiments carried out by other authors have indicated that potassium deficiency leads to a marked reduction in olive yield. Therefore there must be a balance between the NPK fertilizers applied. Figure 5 shows that the average iron, manganese and copper concentrations were significantly higher in bearing years (2000 and 2002) than in non-bearing ones. El-Gazzar et al. (1974) and Gonzalez Garcia and Catalina (1982) reported similar results. It could be concluded that in bearing years there was high removal of these nutrients, leading to low concentrations in non-bearing years.

Discussion

It is a well-known fact that the distribution of roots is related to the amount of irrigation water. Under dry land conditions the installation of a drip system makes the adult tree adapt its rooting system, concentrating the roots within the wet soil zones near the drippers, so that the highest root densities occur in these zones (Fernandez et al., 1991). The roots grow within the limits of the available moisture, making a shallow root pattern common under drip irrigation in arid and semi-arid regions (Ben David, 1975). This results in the development of a more restricted root zone, given frequent fertilizer and water additions.

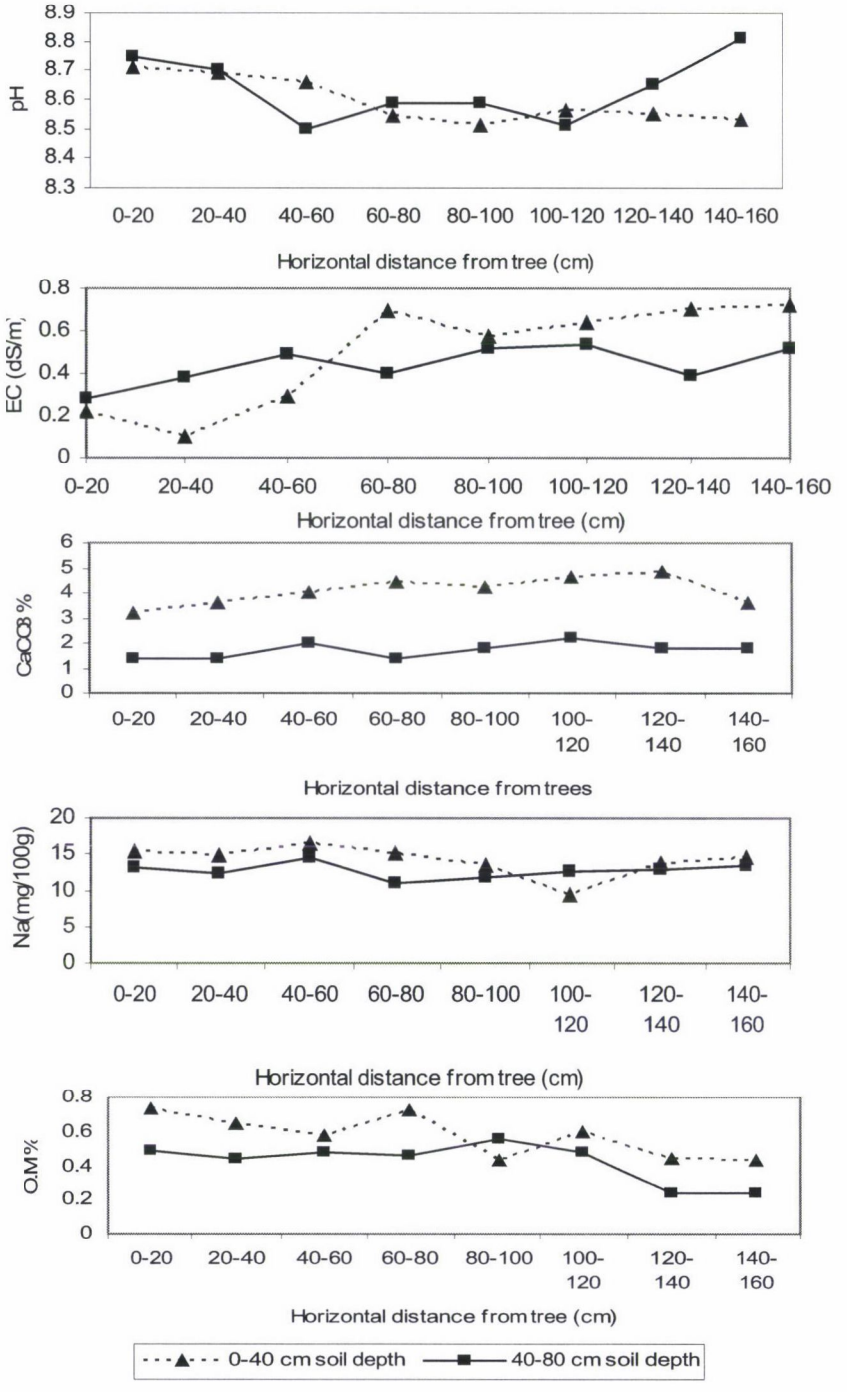

Fig. 1. Properties of soil under olive trees

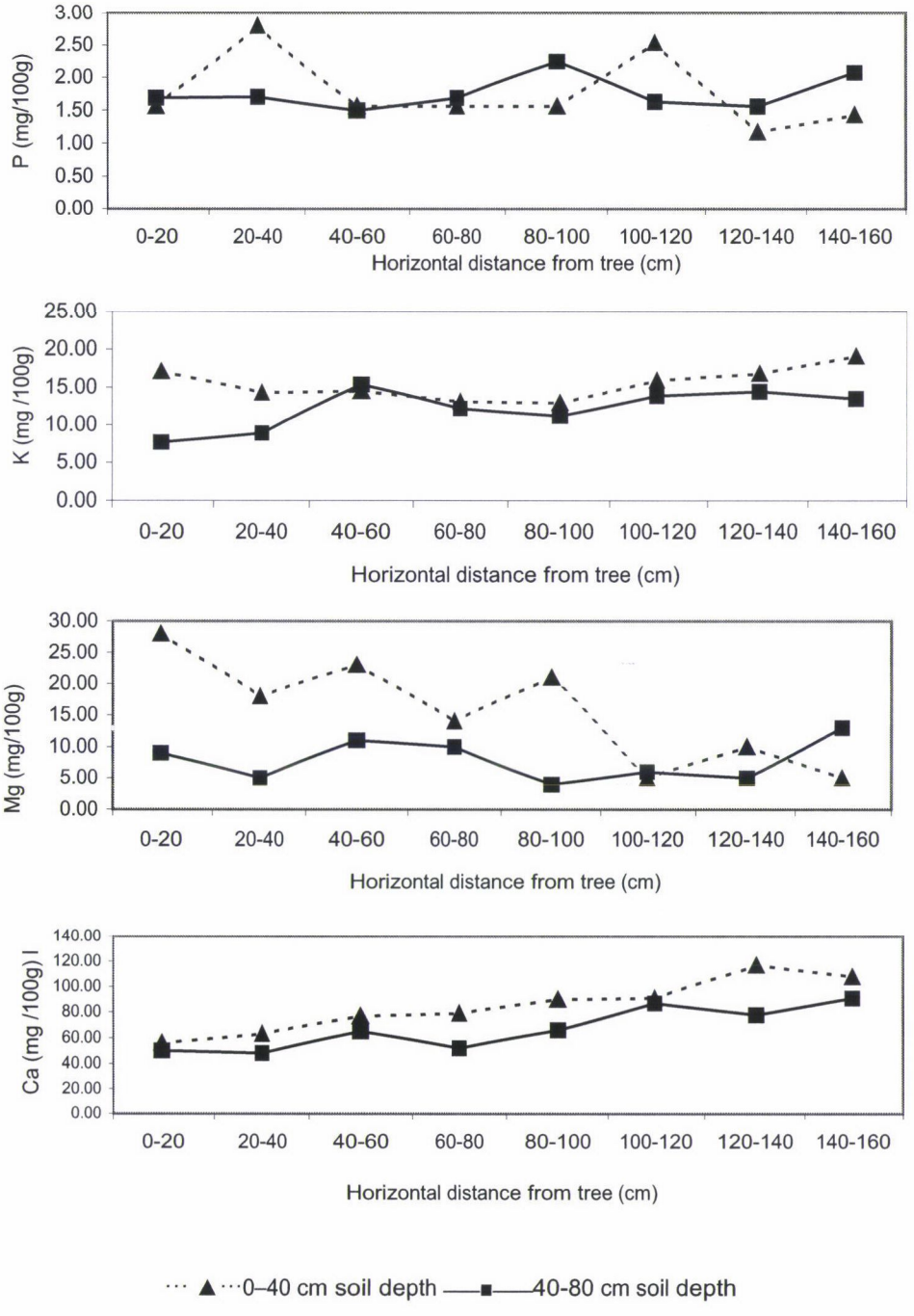

Fig. 2. Horizontal distribution of macronutrients in the soil under olive trees

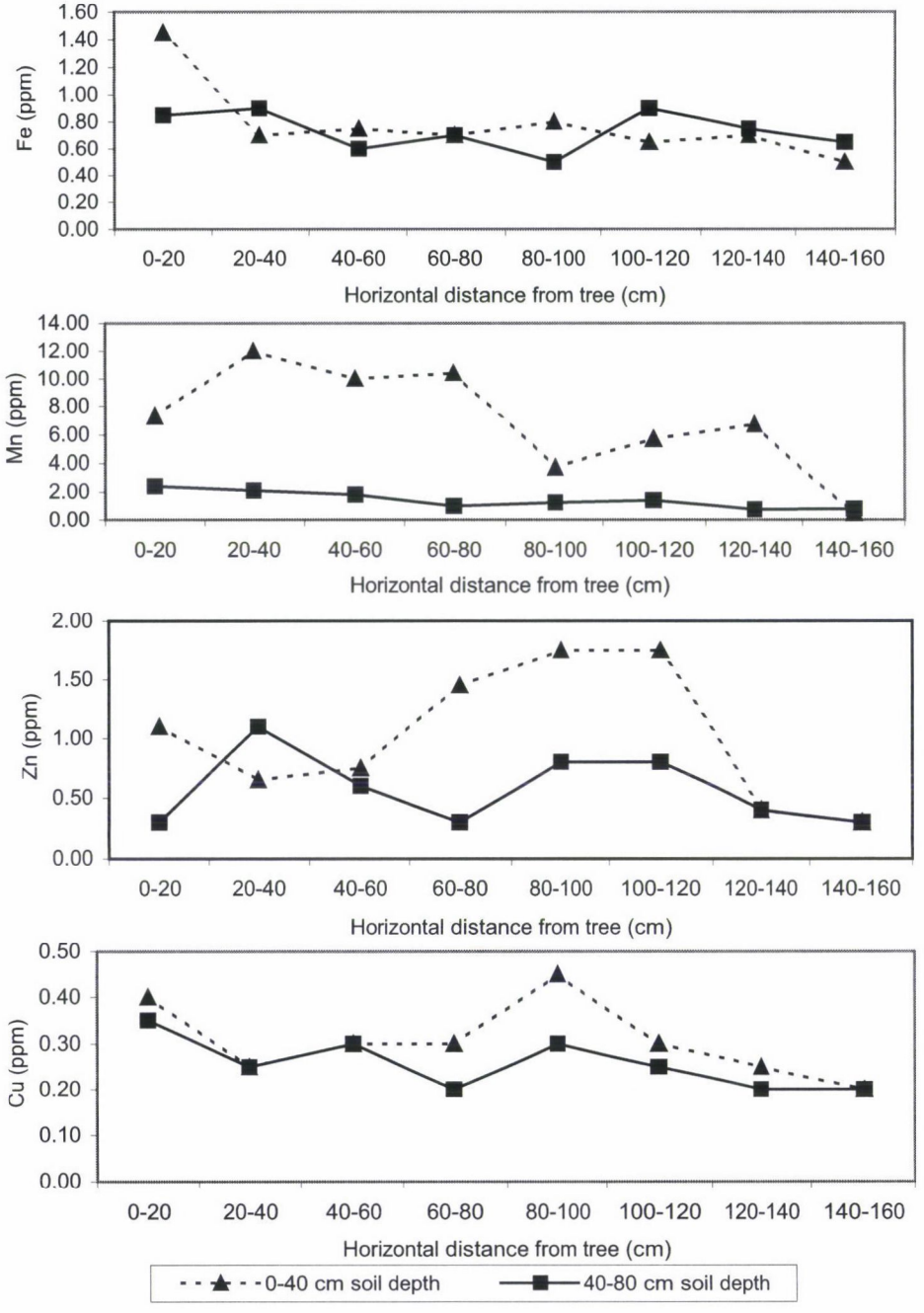

Fig.3. Horizontal distribution of micronutrients in the soil under olive trees

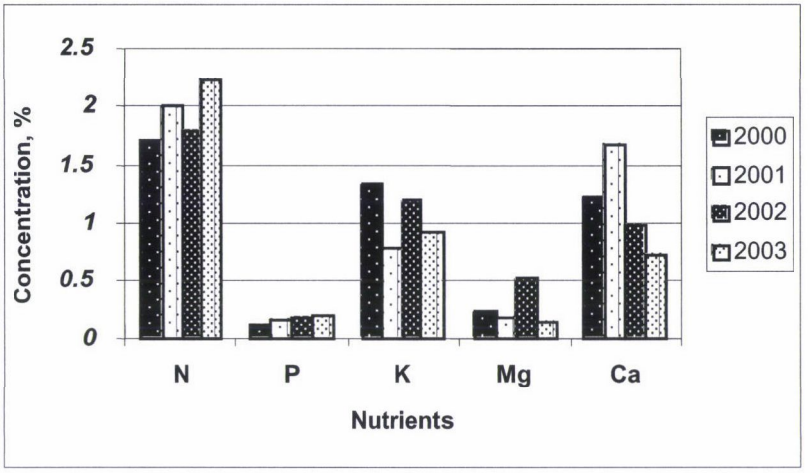

Fig. 4. Macronutrient concentration in leaves of olive trees

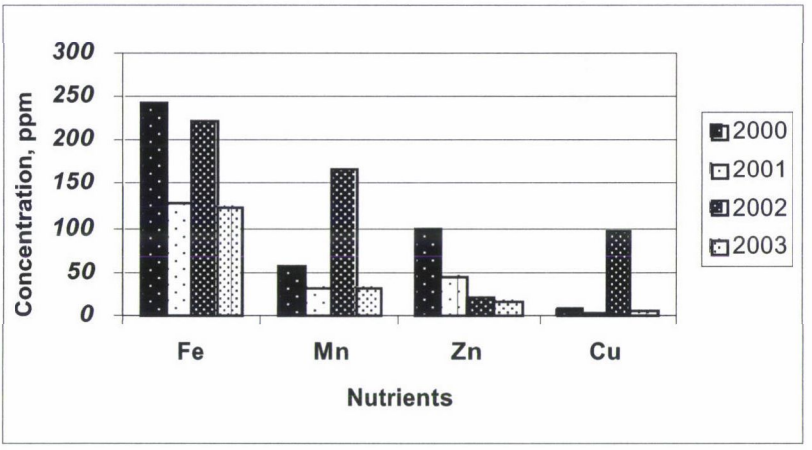

Fig. 5. Micronutrient concentration in leaves of olive trees

Yet the increase in root density within the upper 20–60 cm soil depth indicates the stimulating effect of nutrition. In this connection, Boutros et al. (1988) found a significant increase in the dry weight of fibrous roots in young orange trees after foliar spray with micronutrients.

Values of electrical conductivity and total $CaCO_3$ reflect no salinity or lime problems for olive trees. The high pH value and low organic matter content might create a mobilization problem for phosphorus and micronutrients. The application of organic manure will help to lower soil pH. Optimum soil conditions should be maintained in the root area.

The concentrations of nutrients at various horizontal distances at a vertical depth of 0–40 cm were higher than at 40–80 cm, probably due to the sufficient water volume in the upper layer. To increase the efficiency of fertilizer application, the fertigation system must allow nutrients to be applied where the active roots are concentrated (80–140 cm from the trunk).

The restricted vertical movement of nutrients in the soil confirms this interpretation. The results indicate that nutrient contents may build up in the soil after continuous fertigation. For this reason the concentrations of fertigated nutrients increased in the surface layer, while those of micronutrients decreased with depth, being applied as foliar sprays. Potassium application helped to decrease the uptake of Na from the root area, due to antagonistic effects. The decrease in Na uptake from the root area resulted in lower Na^+ accumulation in the leaves (<0.1%) and appeared to enable the roots to absorb greater amounts of other elements, as shown by the limited decrease in nutrients other than Na in the leaves. This occurred in spite of the pattern of nutrient distribution, which showed that values of Na at most points in the surface soil were higher than in the subsurface soil, due to the effect of evaporation on the surface. A comparison of the leaf samples showed low concentrations of K, Mg, Fe, Mn, Zn and Cu after the bearing year, which was attributed to the fact that flowering and fruiting exhaust the tree considerably (Hartmann et al., 1980). However, the Zn concentration was lower in the third and fourth years. A possible reason for this may be that tree requirements for Zn exceeded the amount added. For this reason it is important to increase the doses of fertilizers containing this element after bearing years to replace its high removal. The recommended fertilizer regime was shown to correct the possible nutrient disorder related to high soil pH, support better growth through the progressive improvement of leaf nutrient status, and result in consistent yield increases between 2000 and 2002. In this connection Liamas (1977) reported that olive trees well supplied with potassium were more resistant to drought and cold. The efficiency of fertilizer use can be increased with fertigation through specific fertilizer recommendations, which lead to improvements in soil fertility and correct any nutrient deficiency occurring during the various stages of growth.

Acknowledgements

This work was conducted as part of the Egyptian-German project "Micro-nutrients and Other Plant Nutrition Problems in Egypt" executed by the National Research Center (NRC) (Coordinator, Prof. M. M. El-Fouly) and the Institute for Plant Nutrition, Technical University, Munich (Prof. A. Amberger). The Egyptian Academy of Scientific Research and Technology (ASRT), and the German Federal Ministry of Technical Cooperation (BMZ) supported the project through the German Agency For Technical Cooperation (GTZ).

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Acta Agronomica Hungarica, 53(3), pp. 349–352 (2005)

Short communication

HEAVY METAL, MACRO- AND MICROELEMENT CONTENT OF GRASS SPECIES AND DICOTYLEDONS

J. TASI

DEPARTMENT OF GRASSLAND MANAGEMENT, CROP PRODUCTION INSTITUTE, SZENT ISTVÁN UNIVERSITY, GÖDÖLLŐ, HUNGARY

Received: 26 May, 2005; accepted: 2 September, 2005

Data on the composition of fodder from pastures (grass, meadow-hay) can be frequently found in the literature, but data on the element contents of the grass or non-grass species composing natural grasslands are scarce. In order to analyse the element contents of plants, samples were collected over 3 years (1998–2000) from different types of natural grasslands. On non-fertilised grasslands, the dominant species was *Festuca pseudovina*. The dominant species on fertilised grasslands was *Poa pratensis*, with *Bromus inermis* or *Elymus repens* being dominant at some sites. The results showed that leguminous plants had the highest contents of four of the five macroelements, while the highest level of K was found in dicotyledonous plants. The Na and Ca contents of the plants were higher on non-fertilised grasslands, while the level of all the other macroelements was higher in plants from fertilised grasslands. Since the Se contents showed a high dispersion, only a trend was observed, indicating that the Se content of grass species is not significantly lower than that of other grassland plants. On non-fertilised grasslands, grass species contained the most Cr and Pb, while leguminous plants contained the most Cd. In fodder from fertilised grasslands, the highest quantity of Cd and Cr was found in grass species, and the most lead in non-leguminous dicotyledons.

Key words: grass species, dicotyledons, macroelement content, heavy metal content

Introduction

Data on the composition of fodder from pastures (grass, meadow-hay) can be frequently found in the literature. Analyses have been carried out to compare regions (Köles et al., 2000) or species (e.g. Genevini and Sciaraffia, 1981). The effect of fertilisation and changes in the composition of grasslands utilised at different times have also been examined (Friedler and Höhne, 1984). The above studies generally refer to sown grass species, or in certain cases to sown dicotyledons. Data relating to the element contents of the grass or non-grass species composing natural grasslands are either scarce or inadequately summarised.

Materials and methods

To analyse the element contents of plants, samples were collected over 3 years (1998–2000) from different types of natural grasslands. The botanical composition of the sampling sites was determined by Balázs's quadrat method (Balázs, 1949). In 1999 and 2000 the plants in all the green samples were divided into groups of grass species, leguminous species and other dicotyledons. The Cd, Cr, Pb, K, Na, Ca, Mg, P and Se contents of each group were measured using a spectrometer.

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The sampling sites can be divided into two categories: fertilised and non-fertilised. The non-fertilised grasslands received no mineral fertilisers, and as these sites were undergrazed, manure from sheepgrazing was of insignificant quantity. In these grasslands, the dominant species was *Festuca pseudovina*. The dominant species in the fertilised grasslands was *Poa pratensis*, though *Bromus inermis* or *Elymus repens* were dominant at some sites.

Results and discussion

Figure 1 shows the average heavy metal contents of grass species, leguminous species and other dicotyledons. The data presented here represent the May measurements.

The analysed grasslands showed a high variation in the heavy metal contents of the plant groups. On non-fertilised grasslands the most Cd was found in leguminous plants, while on fertilised grasslands the most Cd was found in grass species. In general, fertilised grasslands contained more Cd. The toxic standard of 0.5 mg kg^{-1} in fodder was exceeded in each plant group, on both types of grassland. Grass species contained the most Cr on both grassland types. The lowest Cr content was found in leguminous plants on fertilised grasslands. Clover species in the fodder from non-fertilised grasslands accumulated more Cr than the other dicotyledons. The Cr content was higher in the total fodder from fertilised grasslands and exceeded an average plant quantity of 1 mg kg^{-1}. The greatest quantity of lead was absorbed by grasses on non-fertilised grasslands, and by non-leguminous dicotyledons on fertilised grasslands. Except for the grass species, the plants on fertilised grasslands contained more lead than the plants on non-fertilised grasslands. A lead content of 10 mg kg^{-1} or more is considered toxic in grass fodder, but this level was not approached by any of the grassland plant groups.

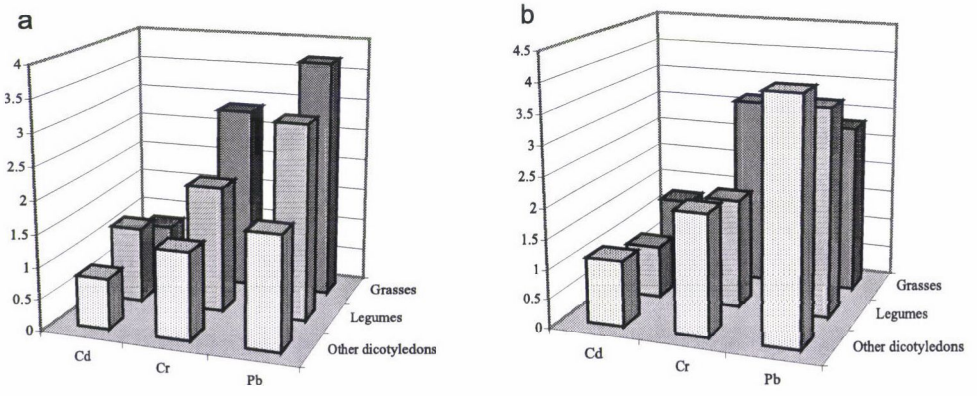

Fig. 1. Average heavy metal content of fodder (mg/kg) on non-fertilised (a) and fertilised (b) grasslands

Table 1 shows the average macroelement content of grass species, leguminous species and other dicotyledons. As expected, these average data showed that grass species contained fewer macroelements than other grassland plants. Leguminous plants contained the highest quantity of four of the five macroelements examined, while K accumulated mostly in other dicotyledonous species. The macroelement content of non-leguminous grassland species was many times that of grasses, but the difference between leguminous and other grassland species was insignificant. The Na and Ca contents of the fodder from non-fertilised grasslands exceeded that of the fertilised grasslands. The elements K, Mg and P were found in greater quantities in fodder from fertilised grasslands than in fodder from non-fertilised grasslands.

The macroelement content of the sampled grass fodders was sufficient to meet the nutritional requirements of sheep.

The Se content of the fodder showed a variation of more than 30% over habitats and years, so only a tendency was observed between fertilised and non-fertilised grasslands. Leguminous plants on non-fertilised grasslands contained more Se than on fertilised grasslands, and there was no significant difference in Se content between grasses and non-leguminous dicotyledons. On fertilised grasslands non-leguminous dicotyledons contained more Se than leguminous plants and grass species. The desirable quantity of Se in bulk fodders is 0.05–2 mg kg^{-1}. The grassland fodders proved to be neither deficient in Se, nor to reach the toxic level of 5 mg kg^{-1}.

Table 1

Average heavy metal, macro- and microelement contents of fodder on fertilised and non-fertilised grasslands (1999–2000)

Element	Units	Grasses	*CV%*	Legumes	*CV%*	Other dicots	*CV%*
Non-fertilised grasslands							
K	mg kg^{-1}	1244.0	*27.7*	18223.3	*10.7*	2344.0	*28.0*
Na	mg kg^{-1}	1989.2	*20.2*	4992.7	*113.3*	3687.0	*29.5*
Ca	mg kg^{-1}	4452.2	*20.5*	14560.0	*31.4*	13162.5	*34.7*
Mg	mg kg^{-1}	1131.7	*19.7*	2873.3	*14.0*	2582.5	*7.7*
P	mg kg^{-1}	2102.5	*18.2*	2763.3	*10.2*	2592.5	*17.8*
Se	µg kg^{-1}	578.5	*74.3*	738.7	*25.6*	546.5	*36.0*
Fertilised grasslands							
K	mg kg^{-1}	18915.0	*9.4*	24353.3	*30.7*	27335.0	*7.8*
Na	mg kg^{-1}	613.2	*16.5*	2698.3	*25.0*	1591.2	*0.6*
Ca	mg kg^{-1}	3470.0	*5.7*	12995.0	*11.9*	10900.0	*8.5*
Mg	mg kg^{-1}	1240.0	*26.2*	4125.0	*4.9*	3602.5	*37.5*
P	mg kg^{-1}	3244.2	*32.4*	3476.7	*13.9*	3550.0	*18.5*
Se	µg kg^{-1}	703.5	*33.3*	648.7	*58.2*	846.2	*45.0*

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Acta Agronomica Hungarica, 53(3), pp. 353–356 (2005)

Short communication

EFFECT OF DIFFERENT LEVELS OF NITROGEN AND PHOSPHORUS ON THE FORAGE AND SEED YIELD OF RYEGRASS (*LOLIUM PERENNE* L.)

K. P. PURI and U. S. TIWANA

DEPARTMENT OF PLANT BREEDING, GENETICS AND BIOTECHNOLOGY, PUNJAB AGRICULTURAL UNIVERSITY, LUDHIANA, INDIA

Received: 2 August, 2004; accepted: 6 May, 2005

Studies were made on the fodder and seed production of ryegrass (*Lolium perenne* L.) using twelve treatment combinations with four levels of nitrogen (30, 60, 90 and 120 kg/ha/cut) and three levels of phosphorus (0, 50 and 100 kg P_2O_5/ha), laid out in a randomized block design with four replications. The mean of two years indicated that nitrogen at 120 kg/ha/cut increased the green fodder (834 q/ha) and dry matter yield (129 q/ha) significantly over the lower doses. After leaving the same crop for seed production during mid-March, nitrogen at the rate of 60 kg/ha gave the highest seed yield (7.61 q/ha). Beyond this dose the seed yield decreased drastically due to lodging. Phosphorus at the rate of 50 kg P_2O_5/ha gave 5% higher dry matter (99.6 q/ha) and 7.4% higher seed yield (7.26 q/ha) over the control.

Key words: ryegrass (*Lolium perenne* L.), nitrogen, phosphorus, seed yield, dry matter

Introduction

Ryegrass (*Lolium perenne* L.) has become an important multicut fodder for the winter season. When sown in the last week of September to the first week of October, it gives 5–6 cuts up to the middle of May. Its total dry matter production is comparable with that of Egyptian clover (*Trifolium alexandrium* L.) and Persian clover (*Trifolium resupinatum* L.), but when it is sown in a mixture with these, the total fodder yield is increased by about 25–30% over Egyptian clover/Persian clover alone and by 10–15% over Egyptian clover/Persian clover + oats (*Avena sativa*) mixture, which is a very popular fodder in India (Chauhan and Puri, 1988; 1992). When sown in a 60:40 mixture with Egyptian clover/Persian clover, ryegrass not only gives higher fodder production, but also provides a balanced fodder for dairy animals.

The preliminary studies conducted by Puri et al. (1989) showed that ryegrass responded well to fertilizers. Added nitrogen increased both vegetative growth and nitrogen content and provided highly digestible forage (Wilman, 1980). The application of phosphorus is equally important because of its effect on root development, which indirectly contributes towards higher fodder production. The application of nitrogen along with phosphorus gives a synergetic effect by increasing vegetative and reproductive growth as well as the quality of ryegrass. The present investigations were carried out to determine the optimum rate of nitrogen and phosphorus for higher forage and seed yield.

0238–0161/$ 20.00

Materials and methods

The research was carried out for two years (2000–2001 and 2001–2002) at Punjab Agricultural University, Ludhiana (India). The experiment was laid out in a randomized block design with four levels of nitrogen (30, 60, 90 and 120 kg/ha/cut) and three phosphorus levels (0, 50 and 100 kg P_2O_5/ha). The soil of the experimental site was low in available nitrogen and phosphorus and medium in potash, with a pH of 8.2. The variety Punjab Ryegrass No. 1 was sown in the first week of October in both years by broadcasting 10 kg seed/ha. The net plot size was 5.5 × 5.0 m each year. Nitrogen in the form of urea was applied in two splits, half at the time of sowing and half as top dressing 21 days after sowing. Phosphorus in the form of single superphosphate was applied at the time of sowing only. The full dose of nitrogen according to the treatments was applied after each cut. The first irrigation was applied immediately after sowing and the second about five days after sowing. Afterwards, the crop was irrigated at an interval of about 10 days, depending upon the prevailing weather conditions. The first cut was taken 55 days after sowing and subsequently at intervals of about one month up to mid-March for fodder production. The same crop was then left for seed production in both years.

Results and discussion

Effect of nitrogen

Increasing levels of nitrogen increased the green and dry matter yield significantly. In each cut, nitrogen improved the fodder yield. On an average of two years, 60, 90 and 120 kg N/ha/cut gave 22.6, 45.1 and 73.2% higher green fodder yield, respectively, over 30 kg N/ha/cut (Table 1). A similar trend was observed for dry matter yield. This may be attributed to the increasing availability and absorption of nitrogen, resulting in more vegetative growth, exhibited as greater tiller height, number of tillers and number of leaves per tiller (Table 2), on account of the enlargement of cells and increased photosynthesis (Singh, 1978), leading to more fodder yield. After leaving the same fodder crop for seed production during mid-March, nitrogen at the rate of 60 kg/ha gave the highest seed yield (7.61 q/ha). Beyond this dose (90 and 120 kg N/ha) the seed yield decreased due to lodging. Nitrogen at 90 and 120 kg N/ha resulted in 9.6 and 11.6% higher lodging, respectively, over 60 kg N/ha. The 1000-seed weight did not differ significantly.

Effect of phosphorus

Phosphorus application increased the green fodder, dry matter and seed yield. The differences between 50 and 100 kg P_2O_5/ha were non-significant for dry matter yield and seed yield. On an average of two years, 50 kg P_2O_5/ha gave 5% higher dry matter (99.6 q/ha) and 7.4% higher seed yield (7.26 q/ha) over the control. The application of phosphorus enhanced root development, which might have increased the tiller height, number of tillers and number of leaves per tiller, and ultimately the fodder yield. A similar trend was observed by Puri et al. (1989). Phosphorus application increased the crude protein content, although the results were non-significant (Table 2). This increase may be due to the intensive root system and the synergetic effect between phosphorus application and other nutrients, including nitrogen, which increases the crude protein content of the

fodder. Phosphorus application increased the 1000-seed weight and decreased lodging, but the differences were non-significant.

It may be concluded that 120 kg N/ha/cut should be applied to ryegrass to obtain higher fodder production up to mid-March, after which 60 kg N/ha seemed to be optimum for higher seed production. Phosphorus at the rate of 50 kg P_2O_5/ha should be applied at the time of sowing.

Table 1

Effect of nitrogen and phosphorus on the fodder and seed yield of ryegrass

Treatment	Green fodder yield (q/ha)			Dry matter yield (q/ha)			Seed yield (q/ha)		
	2000–01	2001–02	Mean	2000–01	2001–02	Mean	2000–01	2001–02	Mean
Nitrogen levels (kg N/ha/cut)									
30	485.5	477.4	481.5	69.5	78.8	74.2	7.03	6.95	6.99
60	590.5	590.0	590.3	85.2	96.6	90.9	7.43	7.78	7.61
90	714.3	683.3	698.8	103.2	110.2	106.7	6.53	7.40	6.97
120	825.4	842.7	834.1	123.2	134.9	129.1	6.43	7.18	6.81
C.D.$_{5\%}$	40.0	95.0	67.5	9.6	22.5	16.1	0.83	0.72	0.78
Phosphorus level (kg P_2O_5/ha)									
0	615.7	620.9	618.3	89.2	100.6	94.9	6.39	7.13	6.76
50	664.1	634.6	649.4	96.4	102.8	99.6	7.16	7.35	7.26
100	682.4	689.8	686.1	100.4	112.0	106.2	7.06	7.50	7.28
C.D.$_{5\%}$	34.7	30.5	32.6	8.4	8.0	8.2	0.19	N.S.	0.30

N.S. = Non-significant

Table 2

Effect of nitrogen and phosphorus on the yield components characters and quality of ryegrass (mean of 2 years)

Treatments	No. of leaves/tiller	Height of tiller (cm)	No. of tillers (0.30 m^{-2})	Crude protein (%)	1000 seed weight (g)	Lodging (on a 0–10 scale)
Nitrogen levels (kg N/ha/cut)						
30	3.71	8.4	121	11.1	1.78	6.09
60	3.79	11.8	149	12.7	1.81	6.49
90	3.88	14.6	156	14.2	1.80	7.08
120	3.92	15.2	163	15.9	1.80	7.21
C.D.$_{5\%}$	0.10	3.22	13.5	2.9	N.S.	0.72
Phosphorus levels (kg P_2O_5/ha)						
0	3.80	12.3	141	13.2	1.79	6.95
50	3.81	12.5	149	13.5	1.80	6.72
100	3.88	12.6	149	13.7	1.81	6.50
C.D.$_{5\%}$	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

N.S. = Non-significant

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Erratum

Regrettably, certain data were omitted from Table 1 of the paper by Rajkai et al., on p. 198 of Vol. 53/2 of Acta Agron. Hung. The complete table is given below.

Table 1
Survey of published root capacitance measurements

References	Plants	Exp. layout	Root medium	Root traits	Plant electrode	Potential (V)	Frequency (kHz)
Chloupek, 1972	Maize	Pot	Quartz sand	Surface area	Needle	12	0.8
	Oat	Pot	Clay soil	Surface area	Needle	12	5
	Onion	Pot	Quartz sand	Surface area	Needle	12	1
	Rape	Pot		Surface area	Needle	12	0.8–5
	Sunflower	Pot	Quartz	Surface area	Needle	12	5
	Sunflower	Pot	Clay	Surface area	Needle	12	5
Chloupek, 1977	Carrot	Field	Loam soil	Carrot biom.	Needle		
	Mustard				Needle	0.1, 1, 10	0.1, 1, 10
	Oat				Needle	0.1, 1, 10	0.1, 1, 10
	Onion				Needle	0.1, 1, 10	0.1, 0.5
	Red clover	Field			Needle		
	Sunflower	Pot	Sand	Mass	Needle		0.8, 5
Chloupek, 1980	Alfalfa	Field	Light soil	*Root system size	Needle		1
Kendall et al. 1982	Alfalfa	Field	Silt loam	Dry m., diam.	Clamp		1
	Red clover	Pot	Hydroponics	Dry m., diam.	Clamp		1
Dalton, 1995	Tomato		Hydroponics	Mass	Needle		1
			Sand		Needle		1
Van Beem et al. 1998	Maize	Pot	Vermicullite	Fresh mass	Clamp		1
	Maize	Field	.Loam	Fresh mass	Clamp		1
Matsumoto et al. 2001	Maize	Pot	Soil	Dry m. length	Needle		N. a.
Ozier-Lafontaine et al.	Maize	Pot	Soil	Dry mass	Needle		1
2001	Carrot	Field	Soil	Dry mass	Needle		1
Rajkai et al. 2002	Sunflower	Pot	Soil	Fresh mass	Needle	1	1
	Sunflower	Pot	Water	Fresh mass	Needle	1	0.3–1000
Preston et al. 2004	Young hyb. poplar tree	Pot	Sand manure and peat mixt.	Dry mass	Clamp		1

*Characterized by the measured electrical capacitance; biom: biomass; Dry m.: Dry mass; N. a.: Not available

For this reason, the following addition should be made to the **References**:

Rajkai, K., Végh, K. R., Nacsa, T. (2002): Electrical capacitance as the indicator of root size and activity. *Agrokémia és Talajtan*, **51**, 89–98.

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Kiss, G., Papp, I., Bakondi-Zámori, E., Gartner-Bánfalvi, Á. (1977): A szója fungicides magcsávázásának és rhizóbium oltásának együttes tanulmányozása. (Joint study of fungicide dressing and rhizobium inoculation in soybean.) *Növénytermelés,* **26,** 147–153.

Ouyang, J. (1986): Induction of pollen plants in *Triticum aestivum.* In: Hu, M., Yang, M. (eds.), *Haploids of higher plants in vitro.* Academic Press, Beijing, 26–41.

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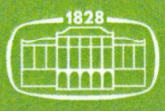
ISSN 0238-0161

Printed in Hungary
PXP, Budapest

Acta Agronomica Hungarica

An International Multidisciplinary Journal in Agricultural Science

VOLUME 53, NUMBER 4, 2005



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ACTA AGRONOMICA HUNG. AAHUEX 53 (4) 359–462 (2005) HU ISSN 0238-0161

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The Agricultural Research Institute of the Hungarian Academy of Sciences contributes financially to the publication of *Acta Agronomica Hungarica*.

AAgr 53 (2005) 4

ACTA AGRONOMICA HUNGARICA

AN INTERNATIONAL MULTIDISCIPLINARY JOURNAL IN AGRICULTURAL SCIENCE

VOLUME 53, NUMBER 4, 2005

AKADÉMIAI KIADÓ, BUDAPEST

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REVIEW

Acta Agronomica Hungarica, 53(4), pp. 359–370 (2005)

DELETION ANALYSIS OF GENES REGULATING COLD- AND PEG-INDUCED CARBOHYDRATE ACCUMULATION IN HYDROPONICALLY RAISED WHEAT SEEDLINGS

G. GALIBA[1], I. KEREPESI[2], J. W. SNAPE[3] and A. VÁGÚJFALVI[1]

[1]DEPARTMENT OF GENETICS AND PLANT PHYSIOLOGY, AGRICULTURAL RESEARCH INSTITUTE OF THE HUNGARIAN ACADEMY OF SCIENCES, MARTONVÁSÁR, HUNGARY
[2]DEPARTMENT OF GENETICS AND MOLECULAR BIOLOGY, UNIVERSITY OF PÉCS, PÉCS, HUNGARY
[3]CROP GENETICS DEPARTMENT, JOHN INNES CENTRE, NORWICH, NORFOLK, U.K.

Received: 30 September, 2005; accepted: 14 November, 2005

The mobilization of carbohydrates, especially sucrose, is considered very important during both the cold acclimation process and water stress, while sugars also promote floral transition and cold hardiness. Chinese Spring (CS) 5AL and 5DL deletion lines were studied for the physical assignment of the gene(s) regulating stress-induced sugar accumulation. To separate the effect of cold from that of water deprivation, the seedlings were raised in hydroponics, and apart from the cold, the effect of PEG-induced water stress was also evaluated in a time course experiment. The genes affecting stress-induced carbohydrate accumulation were assigned to the same chromosomal bins, which contain the vernalization genes *Vrn-A1* and *Vrn-D1*, on the long arms of chromosomes 5A and 5D, respectively. Sugar accumulation was found to be controlled by *Vrn* genes in an epistatic manner at least at the beginning of the cold treatment. In the case of cold treatment, *Vrn-A1* proved to be more effective than *Vrn-D1*, while in the case of osmotic stress the gene assigned to the long arm of chromosome 5D seemed to be more effective at regulating sugar accumulation than its counterpart on 5A.

Key words: carbohydrates, cold acclimation, drought resistance, water stress, vernalization response, wheat

Abbreviations: CS, *Triticum aestivum* ssp. *aestivum* cv. Chinese Spring; Ch, *Triticum aestivum* ssp. *aestivum* cv. Cheyenne; QTL, quantitative trait locus; EST, expressed sequence tags; FL, fraction length; PEG, polyethylene glycol; WSC, water-soluble carbohydrate; FW, fresh weight

Introduction

Plants must be hardened if they are to achieve their full genetic potential for frost tolerance. Temperatures of 2–5°C and photoperiods of about 12 h are considered to be optimal for cold hardening under controlled environmental conditions, although temperatures below 0°C may induce a second phase in the hardening process, leading to the maximal expression of freezing tolerance (Fowler et al., 1983; Kacperska, 1993). During cold acclimation, a series of responses are induced in plants at the cellular, physiological and developmental levels, resulting in the enhancement of frost tolerance.

The effects of cold and water deficit cause very similar symptoms in herbaceous plants. In both, expansion growth decreases together with a marked reduction in leaf water content, which slows the actual rate of photosynthetic CO_2 assimilation and, if the stress is severe, the sucrose content in the leaves

0238–0161/$ 20.00

falls due to low assimilation and continued respiration, plus the synthesis of amino acids (Hurry et al., 2002; Lawlor and Cornic, 2002). However, under moderate water stress conditions, the reduction in organ expansion is often accompanied by increased carbohydrate content, which suggests that the total assimilation rate is less affected than the total demand (Quick et al., 1989; Kerepesi and Galiba, 2000).

Shifting leaves grown at optimal temperatures to chilling conditions results in the severe repression of photosynthesis (Holaday et al., 1992; Strand et al., 1997; 1999). In contrast the leaves of cold-hardy herbaceous plants, such as *Arabidopsis*, wheat and rye, which develop in the cold, fully retain their photosynthetic capacity (Hurry et al., 1998; Strand et al., 1997). This recovery is important because it not only enables the plants to avoid photoinhibition (Hurry and Huner, 1992), but also re-establishes the plant's ability to produce sucrose. Sucrose plays a key role as a cryoprotectant, but is also the starting point for the basic metabolism and, as a source of cellular energy, is essential for plant growth, maintenance and repair processes, and plant survival (Williams et al., 1992).

The genetic control of frost resistance is complex and can be regarded as a polygenic trait (Sutka, 1981). Major QTLs affecting frost tolerance have been mapped on chromosome 5A (*Fr-A1,* formerly *Fr1*; Galiba et al., 1995), 5D (*Fr-D1*, formerly *Fr2;* Snape et al., 1997) and very recently on 5B (*Fr-B1*; Tóth et al., 2003). Vernalization (*Vrn*) genes determine the need for low temperature to induce flower development. Winter-type cereals require vernalization for flower initiation, while spring types do not. Most of the variation in vernalization requirement in hexaploid wheat is controlled by *Vrn-A1* (formerly *Vrn1*) (Law et al., 1976; Dubcovsky et al., 1988). The *Vrn-A1* gene has been mapped in colinear regions on the long arms of chromosomes 5A (Galiba et al., 1995; Dubcovsky et al., 1988), 5B (Tóth et al., 2003; Iwaki et al., 2002) and 5D (Snape et al., 1997).

Vágújfalvi et al. (1999) showed that chromosomes 5A and 5D of the frost-resistant variety Cheyenne (Ch) increased not only the frost tolerance of the recipient frost-sensitive variety Chinese Spring (CS) but also its sugar content in the course of a 51-day cold treatment. The fructan and sucrose contents in 5A chromosome recombinant lines arising from a cross between CS(Ch 5A) and CS(*Triticum spelta* 5A) were determined after cold hardening. It was shown that a gene regulating sucrose accumulation is closely associated with the V*rn-A1* locus but separable from *Fr-A1* (Galiba et al., 1997).

Endo and Gill (1996) reported on the isolation of 436 deletions in the CS genetic background, and recently a set of these deletion stocks has been used in the chromosome bin mapping of ESTs (expressed sequence tags) (Qi et al., 2002). Some of the 5A deletion lines were used for the physical mapping of the *Vrn-A1* and *Fr-A1 (Fr1)* loci (Sutka et al., 1999). The fraction length (FL) value in a given deletion line identifies the breakpoint in the deleted chromosome and the length of the remaining chromosome arm from the centromere relative to the length of the complete arm. The *Vrn-A1* locus was assigned between the

breakpoints FL 0.78 and FL 0.68, while the *Fr-A1* gene was localised proximal to this position, between breakpoints FL 0.68 and FL 0.67 (Sutka et al., 1999). Therefore, it was possible to determine the physical positions of these genes by deletion analysis.

The main goal of this study was the physical assignment of the gene(s) regulating stress-induced sugar accumulation in CS 5A deletion lines. The 5D chromosome also appeared to play a role in osmoregulation and carbohydrate accumulation (Galiba et al., 1992; Vágújfalvi et al., 1999), so two of the CS 5D deletion lines were also evaluated. Chromosome 5B was not found to play a substantial role in osmotic adjustment (Galiba et al., 1992; 1993), so this chromosome was not evaluated in the present experiment. To separate the effect of cold from that of water deprivation, the seedlings were raised in hydroponics and, apart from the cold, the effect of PEG-induced water stress was also evaluated in a time course experiment.

Materials and methods

Plant material

The four Chinese Spring (CS) 5A and two 5D deletion lines (Fig. 1) used in this study were developed by Endo and Gill (1996). All the genetics stocks are maintained at the Wheat Genetics Resource Centre (WGRC), Department of Plant Pathology, Kansas State University, Manhattan, Kansas, USA. Chromosome deletion lines were kindly provided for research purposes by B.S. Gill (Kansas State University, USA). The lines studied were CS del 5AL 6, 8, 10, 15 and CS del 5DL 2 and 5.

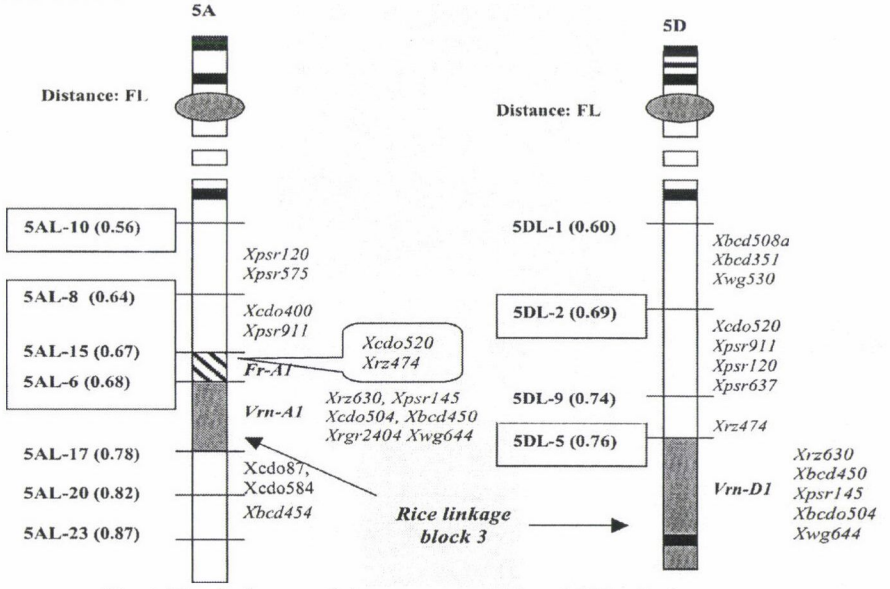

Fig. 1. Physical maps of chromosomes 5AL and 5DL of wheat.
Fraction lengths (FL) of the individual deletion lines are indicated on the left side of the maps. Molecular markers assigning the bins between break points are situated on the right. The deletion lines used in this study are boxed. The maps are based on: Galiba et al., 1995; Snape et al., 1997; Faris et al., 2000; Sutka et al., 1999; Sharma et al., 2000; Boyko et al., 2002

Experimental design and treatments

The seeds were germinated on moist filter papers at room temperature for 2 days. The young seedlings were transferred to perforated plastic boxes containing modified Hoagland solution (Nagy and Galiba, 1995) and were raised in half-strength nutrient solution in a plant growth chamber (Conviron, Ontario, Canada). To avoid anoxia, the roots were aerated using aquarium pumps. The control plants were raised with 16 h illumination (260 μmol s^{-1} m^{-2}) at 18°C and 8 h dark at 13°C throughout the experiment. Moderate osmotic stress was induced at the beginning of the third week by treatment with 15% (w/v) polyethylene glycol (PEG MW 3350), resulting in an osmotic potential of –0.72 MPa, for 39 days. During the PEG treatment the growing conditions were the same as described for the control. Other plants were shifted to +2°C day/night temperature at the same time, with or without 15% PEG application for 37 days. The light intensity and daylength were the same as for the control. The temperature was further decreased on the 37th day to –2°C and the plants were kept under these conditions for 2 days in the dark. Samples of leaf blades were taken after 0, 3, 11, 28, 35 and 39 days of treatment.

Chemical analysis

The total water-soluble carbohydrates and the glucose, fructose, sucrose, fructan and glucan contents were determined on fresh leaves of the plants according to Kerepesi et al. (1996). In brief, samples of 2 g fresh weight were extracted twice under reflux using 40 ml of 80% boiling ethanol for 15 min. The fractions were collected and purified by filtering, dried under reduced pressure at 40°C, and dissolved in distilled water. Oligosaccharides were hydrolysed by boiling in 0.5% HCl for 30 min. The amounts of free and bound glucose, fructose and sucrose were measured using Boehring Mannheim GmbH Kits No. 7160 260. The total water-soluble carbohydrates were determined using the phenol-sulphuric acid method (Dubois et al., 1956). Three replicate samples were taken for each measurement.

Statistical analysis

The experimental design was a randomized complete block with three replications. The data were analysed using the *t*-test and ANOVA functions of the STATGRAPHIC (Statistical Graphics Corporation, Princeton, USA) statistical package to assess significant differences between the means.

Results

Control

Differences in the leaf WSC and sucrose contents were detected between CS and the CS5A and 5D deletion lines (Figs. 2A, E; 3A, E). The deletion lines tended to accumulate more WSC and sucrose over time and these differences became obvious after 11 days of maintenance. The WSC content in the leaves of CS plants proved to be quite stable. It was around 10 mg g^{-1} fresh weight (FW) in the first 11 days and in time it doubled to a value of 20±2.8 (SD) mg g^{-1}, after which it remained constant at this level until the end of the experiment. The changes in WSC content in the 5AL deletion lines showed a curve of the saturation type, reaching a maximum value of around 30 mg g^{-1} on the 11th day and then remaining at this level (Fig. 2A). The behaviour of the 5DL deletion lines was different in this respect. Their WSC content increased in a linear fashion over time, surpassing a value of 80 mg g^{-1} on the 39th day (Fig. 3A). The sucrose content did not show any significant changes over time, staying in the range of 2–10 mg g^{-1} throughout the experiment (Figs. 2E, 3E).

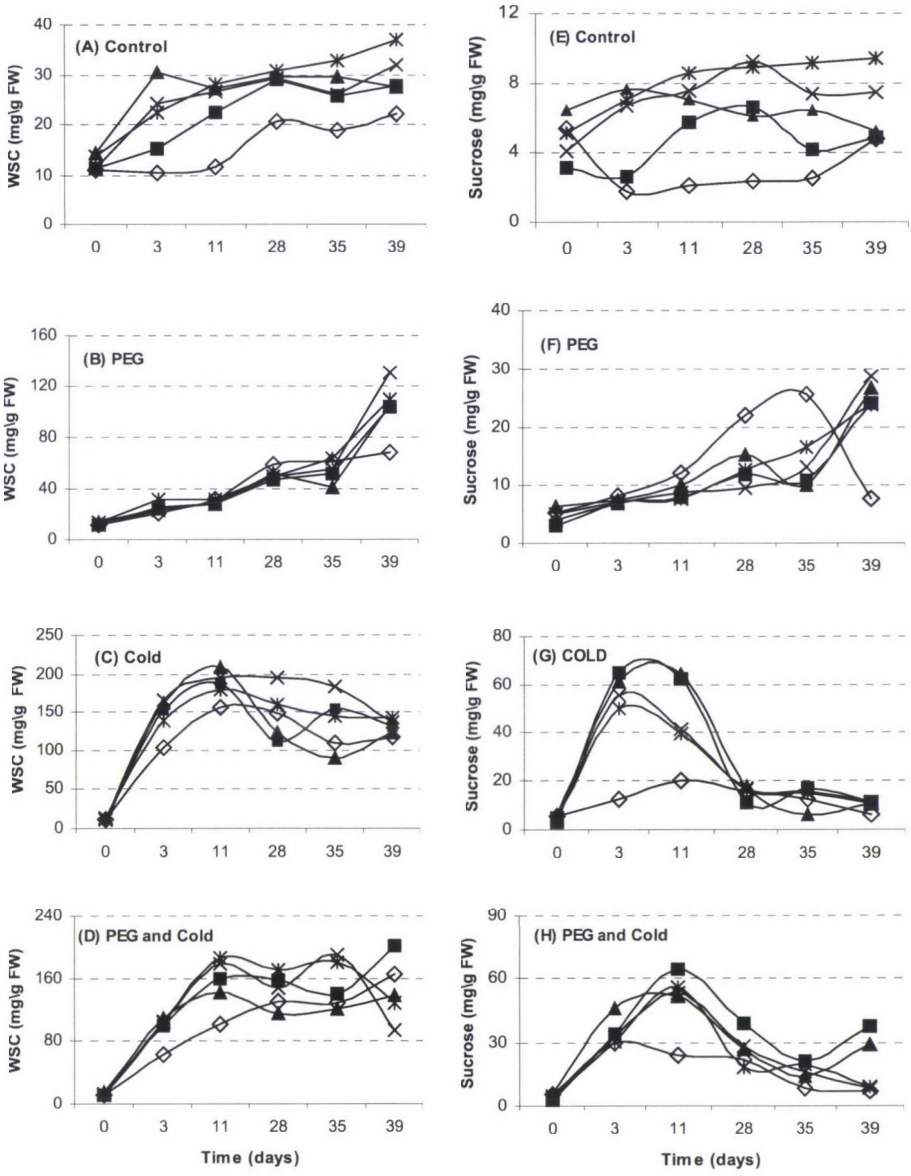

Fig. 2. Time-dependent changes in the total water-soluble carbohydrate (WSC) and sucrose levels in the leaves of Chinese Spring (CS) and CS5AL deletion lines grown in hydroponics. Treatments: Control: 18/13°C day/night; PEG: PEG 15% (–0.72 Mpa); Cold: +2°C for 37 d and –2°C for the last 48 h; Symbols: Chinese Spring (-◊-); CS5AL-6 (-■-); CS5AL-15 (-▲-); CS5AL-8 (-×-); CS5AL-10 (-*-)

PEG treatment

In the case of CS, the WSC content increased two-fold by the third day and three-fold by the 11th day after exposure to PEG-induced mild osmotic stress (–0.72 Mpa), after which it stayed constant until the end of the experiment. The WSC content in the leaves of 5AL deletion lines changed in a similar manner to that in CS until the 35th day of treatment, but the concentration doubled by the 39th day in all the lines (Fig. 2B), with a mean concentration of 112 ±20 mg g^{-1}, which is more than three times the corresponding control value (30±6 mg g^{-1}). The WSC titre in the leaves of the 5DL deletion lines remained at the control level throughout the PEG treatment (Fig. 3B).

A highly significant ($p < 0.001$), ten-fold, transient sucrose accumulation was observed in CS between the 28th and 35th days of treatment, while in the 5AL deletion lines a significant ($p < 0.05$) two-fold accumulation occurred later, on the 39th day (Fig. 2F). As in the case of the WSC content, the sucrose content of the 5DL deletion lines was not affected significantly by PEG application (Fig. 3E, F).

Cold treatment

In all the genotypes studied, the WSC content changed in a very similar manner after exposure to +2°C. A sudden 7–10-fold increase was observed after three days, while further accumulation took place until the 11th day of treatment, reaching maximum values of 150–200 mg g^{-1} (Figs. 2C, 3C). The rate of WSC accumulation was significantly higher in the 5AL deletion line by the third day than in either CS or in the 5DL deletion lines ($p < 0.1$). The most striking differences between the sucrose contents of CS and of the 5AL deletion lines were observed up to 11 days of cold treatment (Fig. 2G). In the 5AL deletion lines highly significant transient sucrose accumulation was observed and roughly 6 times more (about 60 mg g^{-1}) sucrose accumulated than in CS. Transient sucrose accumulation was also observed in the 5DL deletion lines, but the rate and duration of the accumulation was much lower than in the 5AL lines (Fig. 3G). The two-day treatment at –2°C in the dark did not cause any significant alteration either in the rate of sugar accumulation or in the composition of the water-soluble carbohydrates (not shown) compared to the effect of +2°C.

Combined PEG and cold treatment

As a result of combined treatment, the WSC titre in CS increased gradually throughout the experiment, though the rate of accumulation was less intensive than in the case of cold treatment alone. As in the previous case, when cold was applied alone, the WSC titre was higher in the 5AL deletion lines than in CS at the beginning of the experiment (Fig. 2D), but as in CS, the rate of accumulation was lower than when cold was applied alone. In the case of 5DL deletion lines, the rate of WSC accumulation was not affected by PEG application, so the WSC contents significantly exceeded the values measured for CS at the same time (Fig. 3D).

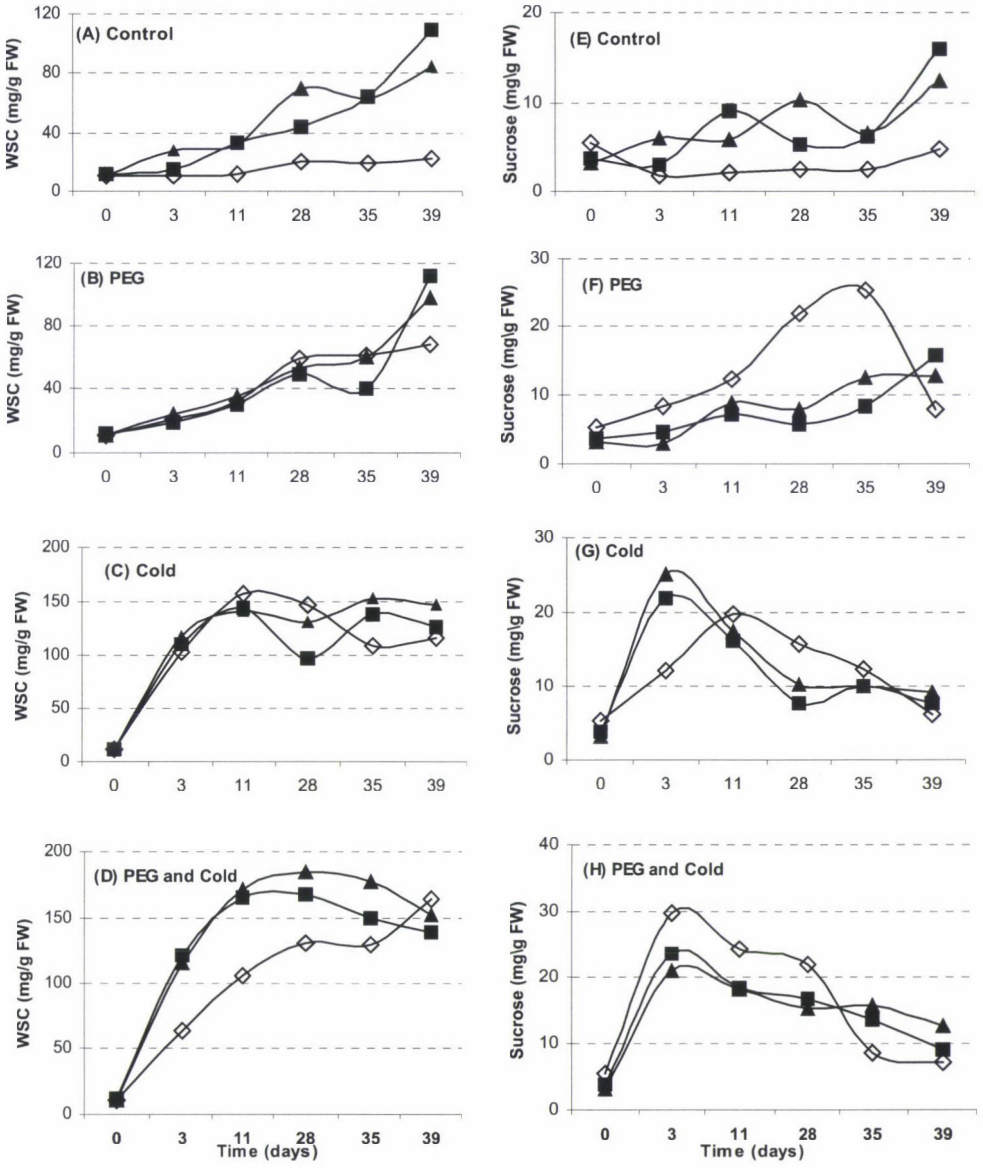

Fig. 3. Time-dependent changes in the total water-soluble carbohydrate (WSC) and sucrose levels in the leaves of Chinese Spring (CS) and CS5DL deletion lines grown in hydroponics. Treatments: see caption to Fig. 2. Symbols: Chinese Spring (-◊-); CS5DL-5 (-■-); CS5DL-2 (-▲-)

Discussion

Measurements on the WSC and sucrose concentrations in the 5AL and 5DL deletion lines (Figs. 2, 3) clearly showed that their sugar contents were similar to each other but differed from that of CS, irrespective of the type of stress applied. It can thus be concluded that the position of the alleles regulating sugar accumulation must be distal to the breakpoint FL 0.68 on 5AL and to FL 0.76 on 5 DL (Fig. 1).

It is possible to determine the physical location of the regulatory gene on 5AL more accurately by comparative mapping in terms of rice linkage blocks. The wheat homoeologous group 5 chromosomes were characterized physically in terms of rice linkage blocks using a deletion mapping approach (Sharma et al., 2000). Nine markers were mapped on chromosome 5A to the chromosome bin located between breakpoints 0.68 and 0.78, among which seven markers were mapped on the long arm of rice chromosome 3 (Causse et al., 1994; Harushima et al., 1998). Thus, this deletion region can be considered as showing conserved synteny with the distal one-third of the long arm of rice chromosome 3. Similarly, the chromosomal region distal to breakpoint 0.76 on the long arm of chromosome 5D can be considered as showing conserved synteny with the long arm of rice chromosome 3 (Sharma et al., 2000). There is thus conserved synteny between the segment of chromosome arm 5AL between the breakpoints 0.68 and 0.78 and the region distal to breakpoint 0.76 on the 5DL arm (Fig. 1). The physical mapping of *Vrn-A1* and *Vrn1-D1* genes into syntenious chromosome bins (Sharma et al., 1988; 2000; Sutka et al., 1999) indicates that some functional synteny can be predicted from the structural synteny.

The *Fr-A1* gene was mapped physically to a chromosomal region proximal to breakpoint 0.68 (Sutka et al., 1999). It is thus clear from the presented results that the gene regulating stress-induced sugar accumulation is situated in the same chromosome bin as *Vrn-A1*, distal to *Fr-A1*. Previously, using chromosome 5A recombinant lines, it was found that the gene regulating sucrose accumulation co-segregated with *Vrn-A1*, but could be separated from *Fr-A1* by recombination (Galiba et al., 1997). The present work confirms this result by physical mapping.

A growing amount of evidence supports the substantial role of carbohydrates in the induction of flowering (Bernier et al., 1993; Corbesier et al., 1998; Zhuo et al., 1998). Contradictory results have been published on the positive or negative effects of high and low concentrations of sugars on floral transition. However, it has been clearly demonstrated that sucrose induces flowering by increasing *LFY* expression in *Arabidopsis* plants (Blazquez et al., 1998). A study on winter rye (variety Petkus) indicated that the first stage of vernalization requires sugar, while the second phase does not. It is, however, dependent upon oxygen (Leroy, 2003). Considering the substantial differences found in the sucrose contents of CS and the CS5AL deletion lines during the

beginning of cold treatment (Fig. 2G) it is plausible to assume that a cold-inducible transcription factor is responsible for the regulation of sugar content during cold acclimation. It is reasonable to hypothesize that *Vrn* genes could serve this purpose. *Vrn-A1* is most likely a MADS-box gene similar to the *Arabidopsis* meristem identity gene *AP1* (Danyluk et al., 2003; Yan et al., 2003). The transcription of *Vrn-A1* is regulated by cold treatment and its expression should affect the expression of downstream alleles regulating sugar accumulation, located either on chromosome 5A or on other chromosomes (Vágújfalvi et al., 1999), in an epistatic manner. It can be seen that in the 5A and 5D deletion lines, the absence of *Vrn-A1* results in considerably greater sugar accumulation than that of *Vrn-D1* (Figs. 2C, G; 3C, G). This is in accordance with the accepted view that vernalization is controlled mainly by *Vrn-A1*, the absence of which cannot be easily compensated for by other *Vrn* genes (Snape et al., 2001; Danyluk et al., 2003; Yan et al., 2003).

Other possible candidate genes possibly affecting sugar accumulation could be the *CBF* genes (C-repeat Binding Factor). These are the key determinants of both low-temperature and drought-stress resistance in *Arabidopsis* (Thomashow et al., 2001). The map locations of barley and wheat *CBF* genes have already been determined, and in both species they are clustered proximally to the *VRN* region (*Vrn-H1* and *Vrn-A1*, respectively) in an orthologous position beside the *psr911* RFLP marker on the long arm of chromosome 5 (Choi et al., 2002; Vágújfalvi et al., 2003; Francia et al., 2004). However the location of the *psr911* marker is proximal to the bins containing either *Vrn-A1* or *Fr-A1* genes (Fig. 1) this hypothesis is unlikely to be valid.

Apart from the effect of cold, the behaviour of the 5AL and 5DL deletion lines was also different under mild osmotic stress. Unlike the 5A deletion lines, the 5D lines lost their ability to accumulate sugar (Fig. 3B, F). This suggests that they have lost an important regulatory allele responsible for sugar accumulation during water deprivation.

In summary, this paper discusses the physical location of genes regulating stress-induced carbohydrate accumulation during cold stress and mild osmotic stress. Sugar accumulation, as a phenotype, is expressed independently of the *Fr* genes, at least at the beginning of cold treatment, and has been shown to be controlled by *Vrn* genes behaving in an epistatic manner. From this aspect, *Vrn-A1* was found to be more effective than *Vrn-D1*. The puzzle of the relationship between the carbohydrate regulatory genes and the *Vrn* genes can only be elucidated by the fine mapping of this interesting chromosomal region. The allele assigned to the long arm of chromosome 5D seems to be more effective at regulating osmotic stress-induced sugar accumulation than its counterpart on 5A.

Acknowledgements

This work was supported by OTKA T046573 and NKFP4/038, Marton-3.2 (Wheat Spike Consortia).

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Acta Agronomica Hungarica, 53(4), pp. 371–376 (2005)

STUDY OF THE EARLY GROWTH AND TILLERING CAPACITY OF BARLEY (*HORDEUM VULGARE* L.) UNDER CHANGING TEMPERATURE AND LIGHT CONDITIONS IN A GRADIENT GROWTH CHAMBER

B. KŐSZEGI, I. KARSAI, K. MÉSZÁROS and G. KOVÁCS

AGRICULTURAL RESEARCH INSTITUTE OF THE HUNGARIAN ACADEMY OF SCIENCES, MARTONVÁSÁR, HUNGARY

Received: 10 March, 2005; accepted: 14 November, 2005

The early growth and tillering capacity of two barley (*Hordeum vulgare* L.) varieties (Dicktoo and Kompolti Korai) were investigated in a gradient growth chamber. The identification of these crop traits is important under organic agricultural conditions in the selection of new varieties for competitiveness against weeds. The results clearly demonstrate that the initial development of the two barley varieties depended considerably on the plant growth conditions. The temperature gradient was found to have the greater effect during early development, causing significant differences in all the traits at all measurement dates. The results indicate that the two varieties differ substantially for two characteristics important for organic farming. As regards tillering ability, Dicktoo appears to be the more desirable type, despite the fact that it is unable to achieve its tillering potential at higher temperatures. Under certain ecological conditions, the relative temperature insensitivity of Kompolti Korai could be an advantage. As far as early development vigour is concerned, Kompolti Korai is clearly a desirable type for organic farming, since it produced rapidly growing, robust plants in all the temperature ranges. From the point of view of organic breeding, a combination of the valuable traits of these two varieties could be the way forward.

Key words: barley, gradient plant growth chamber, early development, tillering capacity

Introduction

The selection of new barley varieties for competitiveness against weeds under organic conditions requires the identification of crop traits and the development of routine methodologies to indicate their potential usefulness. Although some barley varieties have higher weed suppression than others, this is usually not attributed to a single characteristic, either within or between varieties. The interaction between a series of desirable characteristics is important is weed competition (Eisele and Köpke, 1997) and this includes strengths in some characteristics compensating for weaknesses in others. Certain key characteristics are indicated as generically desirable for organic barley varieties to improve weed suppression: (1) good establishment ability, (2) high tillering capacity, (3) increased plant height (Didon and Hansson, 2002), (4) planophile leaf habit and high leaf area index (Seavers and Wright, 1999), (5) early plant growth habit and leaf inclination (Lemerle et al., 1996). Many individual plant traits can be used to define plant growth habits. These characteristics also determine whole crop parameters such as leaf canopy size and light interception. Thus, ground cover also comprises a broad range of plant characteristics (Didon and Hansson, 2002).

0238–0161/$ 20.00

High tillering capacity is likely to be the most important trait in weed suppression for low plant density populations. As organic seeds are not treated for diseases and pest control, establishment in adverse conditions may be reduced significantly (Hoad et al., 2005). Therefore, high, consistent tillering capacity across a range of environmental conditions is an important requirement for organic varieties. Rapid early growth allows the crop to maintain a light interception lead over the rapidly growing weeds, and, if it has the right habit, to shade newly emerging weeds. Early ground cover by the crop is strongly correlated with weed suppression up to full canopy cover. In continental regions rapid autumn and spring growth is particularly important to shade the weed flora that largely emerges in autumn and spring. Although the heritability of tillering capacity and early plant growth is relatively high, the genotype × environment interaction is highly significant, and may modify the performance of both individual plants and crop populations.

The aim of the present study was to determine the effect of different temperatures and light intensities on the early growth and tillering capacity of different barley varieties under inhomogeneous growth conditions in a gradient phytotron chamber.

Materials and methods

The experiments were carried out on two barley (*Hordeum vulgare* L.) varieties, Dicktoo and Kompolti Korai. The seeds of the two varieties were soaked in water at 20°C for 12 hours and then germinated at room temperature for 5 days. After germination the young seedlings were planted into pots, using the method generally employed for plant growth in the phytotron (Tischner et al., 1997). The pots were then placed in the gradient chamber in the Martonvásár phytotron, with the varieties in alternate rows (Tischner and Veisz, 1996). A temperature gradient of 8–18°C was adjusted across the rows and a light intensity gradient of 210–540 μmol m^{-2} s^{-1} across the columns, with a daylength of 16 hours (Kőszegi and Kovács, 2003).

Tillering capacity was recorded three times for each plant during the experiment. The early plant growth was evaluated on the basis of the green and dry biomass production of each individual plant.

Results and discussion

The results clearly demonstrate that the initial development of the two barley varieties depended to a considerable extent on the plant growth conditions. During the early stages of development the temperature gradient was found to have the greater effect, causing significant differences in all the traits at all measurement dates (Table 1). Changes in the light intensity only had a significant effect on the water content of the developing seedlings, based on the results of combined analysis of variance, but considerable differences were observed between the two varieties. The tillering ability and initial development of Dicktoo proved to be practically unaffected by an increase in the light intensity, while it responded sensitively to temperature change. By contrast, temperature had a relatively minor effect on the responses of Kompolti Korai, which were influenced to a greater extent by light intensity.

Table 1
Partitioning of the total variance between light intensity (LI) and temperature gradients (TG) in the complete experiment and separately in the two barley varieties, based on two-way ANOVA

Traits	Dicktoo		Kompolti Korai		Complete experiment	
	LI (%SS)	TG (%SS)	LI (%SS)	TG (%SS)	LI (%SS)	TG (%SS)
Tillering 3rd week	2.1	86.8***	4.5	75.1***	2.2	71.1***
Tillering 4th week	2.4	86.4***	4.8*	78.4***	2.9	72.6***
Tillering 5th week	3.0*	85.6***	6.0*	70.9***	2.1	61.9***
Green plant biomass (g)	1.3*	93.2***	2.7**	89.1***	2.4	79.9***
Dry plant biomass (g)	3.2***	90***	5.3***	85.9***	4.8	73.3***
Water content (%)	30.3***	18.5**	35.0***	17.5**	23.8**	6.5

The process of tillering in the two varieties is illustrated in detail in Figure 1. The results reveal that there was no significant difference between the tillering responses of the two varieties in the third week, though differing trends could be observed in the case of temperature. The tiller number in Dicktoo increased continuously, i.e. the higher the temperature, the larger the number of tillers. By contrast, the data for Kompolti Korai suggested that the genotype behaved differently in different phases of the temperature gradient. Between 8 and 13°C the plants responded to higher temperature with an increase in tiller number, while there was no difference in the tiller number between 13 and 16°C. Above this temperature, the tiller number rose again. This led to a significant difference in the tillering capacity of the varieties over a considerable part of the temperature range (13–16°C), while the difference was not significant at the highest and lowest temperatures. In the fourth week this threshold temperature of 13°C could still be observed for Kompolti Korai, as there was again no difference in the number of tillers over the temperature range 13–16°C. The data recorded in the fifth week reflect the actual tillering ability of the two varieties and its temperature dependence. It is clear from the data that Dicktoo has significantly better tillering potential than Kompolti Korai, but the manifestation of this trait depends to a great extent on the growth temperature.

In the medium temperature range (12–16°C) Dicktoo is able to produce substantially more tillers than Kompolti Korai (16 tillers/plant, compared with 13), while below 12°C and above 16°C the genetic differences between the varieties are not manifested. The tillering ability of Dicktoo increased progressively from 10°C, reaching a maximum at 14°C. At higher temperatures the tillering ability gradually declined. It thus appears that the optimum temperature for tillering in this variety is 14°C. In the case of Kompolti Korai, on the other hand, tillering gradually increased up to 13°C, after which there was practically no change. This variety did not respond to relatively higher temperatures.

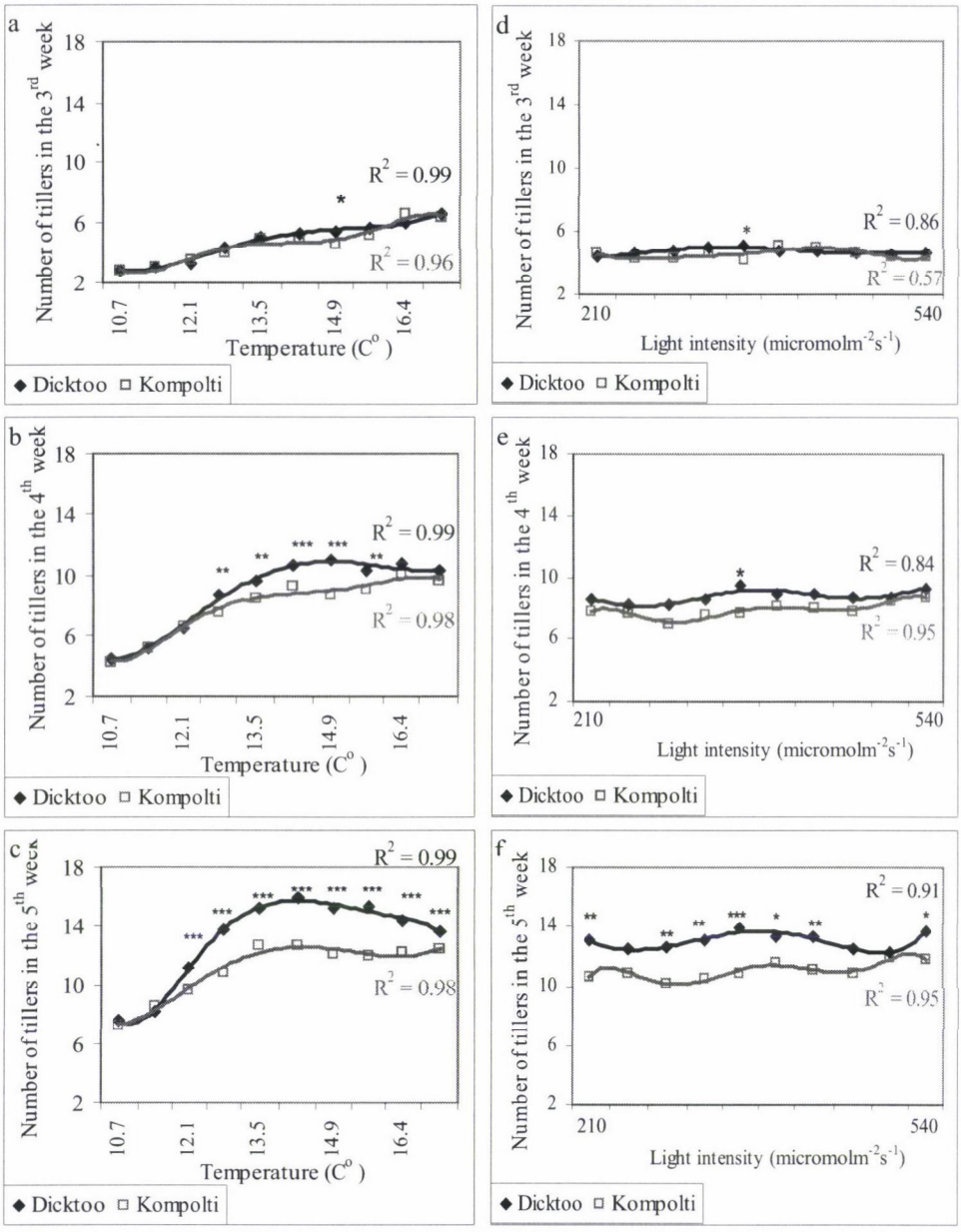

Fig. 1. Effect of temperature (a, b, c) and light intensity (d, e, f) gradients on the tillering capacity of the two barley varieties in three successive weeks (differences between the varieties are significant at the * P=0.05; ** P=0.01 and *** P=0.001 levels)

Changes in light intensity had little effect on tillering in the 3rd and 4th weeks, though by the 4th week there were already signs that Kompolti Korai behaved differently to Dicktoo. Data recorded in the 5th week indicated significantly different responses to light intensity in the two varieties, though the trends were very similar for both. On the basis of earlier studies (Kőszegi and Kovács, 2003) it was not expected that light intensity would have any effect on tillering ability, but the present data suggest that it may play an important role in the expression of this trait. In some intensity ranges the effect of light appeared to be considerable, but it is not yet clear whether this effect was produced by light intensity alone, or whether it could be attributed to a temperature × light interaction.

The differences in the tillering data show that of the two varieties Dicktoo has better tillering ability, but the expression of this potential depends to a great extent on the growth conditions. Kompolti Korai generally has poorer tillering ability, but under some environmental conditions it is capable of producing just as many tillers as Dicktoo.

The biomass production of the two varieties during initial development under the given environmental conditions is presented in Table 2. The data suggest that Kompolti Korai is capable of producing substantially more biomass than Dicktoo throughout the temperature range. Considering that this significantly higher biomass is achieved from significantly fewer tillers, it is clear that the initial development vigour of Kompolti Korai is far better than that of Dicktoo. This difference was observed throughout the temperature range, becoming more pronounced as the temperature rose. Surprisingly, no difference was found between the varieties for water content.

Table 2

Green and dry plant biomass and water content of the two varieties over the temperature gradient

Temperature (C°)	Green plant biomass (g)		Dry plant weight (g)		Water content (%)	
	Dicktoo	Kompolti Korai	Dicktoo	Kompolti Korai	Dicktoo	Kompolti Korai
10.7	5.3	6.5	1.8	2.3	84.8	83.8
11.4	5.6	7.7	1.7	2.2	84.6	84.4
12.1	7.2	9.0	1.7	2.0	85.0	84.2
12.8	10.5	11.8	1.6	2.0	84.6	83.9
13.5	13.2	16.3	2.0	2.8	85.0	83.0
14.2	14.3	17.0	2.2	2.9	84.7	82.7
14.9	14.8	16.9	2.3	2.9	84.2	83.0
15.6	14.0	17.9	2.2	3.0	84.1	83.3
16.4	15.4	18.7	2.5	3.1	83.6	83.6
17.1	16.5	20.5	2.7	3.3	83.7	83.7
$LSD_{0.05}$	1.0		0.6		0.9	

The results indicate that the two varieties differ substantially for two characteristics important for organic farming. As regards tillering ability, Dicktoo appears to be the more desirable type, despite the fact that it is unable to achieve its tillering potential at higher temperatures. Under certain ecological conditions, the relative temperature insensitivity of Kompolti Korai could be an advantage. As far as early development vigour is concerned, Kompolti Korai is clearly a desirable type for organic farming, since it produced rapidly growing, robust plants in all the temperature ranges. From the point of view of organic breeding, a combination of the valuable traits of these two varieties could be the way forward. As both of these varieties are used as models in genetic research and a mapping population has been produced from their cross, it is planned to analyse these traits at molecular level in order to select lines combining the positive traits of both varieties.

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Acta Agronomica Hungarica, 53(4), pp. 377–384 (2005)

EFFECT OF PLANT HORMONES ON THE tRNA ISOACCEPTOR SPECTRUM OF WHEAT

D. SZEGŐ, E. PÁLDI[1], N. B. LOC and D. LÁSZTITY

DEPARTMENT OF PLANT PHYSIOLOGY, EÖTVÖS LORÁND UNIVERSITY, BUDAPEST, HUNGARY
[1]AGRICULTURAL RESEARCH INSTITUTE OF THE HUNGARIAN ACADEMY OF SCIENCES, MARTONVÁSÁR, HUNGARY

Received: 30 May, 2005; accepted: 8 November, 2005

The plant hormones auxin, cytokinin and gibberellic acid, which stimulate plant growth and development, induce significant changes in the isoacceptor spectra of various tRNAs. The present experiments revealed that the treatment of wheat seedlings with auxin, cytokinin or gibberellic acid resulted in the appearance of new isoacceptors in the spectra of three tRNA groups specific for amino acids (methionine, tyrosine and valine). These new isoacceptors may be beneficial for the synthesis and regulation of the proteins induced by the plant hormones.

Key words: plant hormones, tRNA isoacceptors, RCP-5 chromatography, growth, wheat

Introduction

Studies on various types of organisms have proved that changes take place in the RNA content, the RNA composition and modification, the quantity of tRNAs and the composition and mutual ratio of isoacceptor tRNAs specific for the same amino acids under various physiological and pathological conditions (Dunn et al., 1978; Guillemaut et al., 1975; Rácz et al., 1978; 1982; 1987; Tamura et al., 1994). Changes were observed in the tRNA population in the course of embryogenesis and differentiation (Anderson and Cherry, 1969; Bick et al., 1970; Hiatt and Snyder, 1973), after modifications in growth conditions (nutritional status, light, low temperature, etc.) (Wilson et al., 1968; Rácz et al., 1979; 1981; Barnett et al., 1969; Yang and Brown, 1974), in cancerous cells, or after viral infection (Sueoka and Sueoka, 1968; Cornellis and Claessen, 1975). Many authors have reported on deviations in the isoacceptor spectra of tRNAs in different cells of the same organism. Substantial differences were detected, for example, in the $tRNA^{Ser}$ pools of brain and liver tissues in cattle and rabbits (Hatfield and Portugal, 1970). Considerable changes were observed in the $tRNA^{Ser}$ and $tRNA^{Leu}$ isoacceptors on the chromatograms of tRNAs isolated from the liver, spleen and kidneys of mice (Mushinski, 1971). Differences in the $tRNA^{Asp}$, $tRNA^{Ile}$, $tRNA^{Leu}$ and $tRNA^{Lys}$ isoacceptors were reported after comparing the tRNAs of the muscles and eyes of calves (Ortwerth, 1971).

In tissues specialising in the synthesis of specific proteins, major differences were also observed in the tRNA ratio (Garel et al., 1971; Viotti et al., 1978; Pascoe and Ingle, 1978; Duchêne et al., 2001). The changes so far detected in the isoacceptor tRNA spectrum of plant tissues could generally be associated with the differentiation process. Differences were observed in the

ratio and number of isoacceptors for $tRNA^{Leu}$ and $tRNA^{Tyr}$ in the cotyledons and seedlings of soybeans (Bick et al., 1970), for $tRNA^{Lys}$ in the cotyledons and seedlings of lupins (Augustyniak and Pawelkiewicz, 1978), for $tRNA^{Lys}$, $tRNA^{Pro}$ and $tRNA^{Ser}$ in the embryos and seedlings of wheat (Vold and Sypherd, 1968; Walden et al., 1982) and in $tRNA^{Leu}$ in the cotyledons and leaves of pea (Patel and Pillay, 1976). When examining the tRNA RPC chromatogram of pea roots, Vanderhoef and Key (1970) were able to detect a numerical difference in dividing and non-dividing cells for $tRNA^{Tyr}$. Merrick and Dure (1972) discovered changes in the isoacceptor ratio when comparing eight tRNAs in the seed, germ, root, etiolated and green plants of cotton during seed germination. The size, composition and modification of the RNA pool have a considerable influence on translation processes (Lásztity et al., 1991; 1999).

Very few data are currently available on changes in the isoacceptor tRNA composition in plants. The present work examined how the isoacceptor spectra of methionine, tyrosine and valine tRNAs in wheat were affected by growth-stimulating plant hormones, which cause considerable changes in gene expression and thus presumably influence the isoacceptor composition of the tRNA pool due to changes in the codon frequency of the translated mRNAs.

Materials and methods

Eight-day-old seedlings of the wheat (*Triticum aestivum* L.) variety Martonvásári 15 (Mv 15) were used in the experiments. The green plants were grown for three days in the dark on 0.5 mM L^{-1} $CaSO_4$ solution and then in the light on Knop solution. The light intensity was 7000 lux and the duration of illumination 17 hours. Plants in the hormone treatments were grown on Knop solution containing 10^{-6} mol L^{-1} gibberellic acid (GA_3), kinetin or auxin after being transferred to the light.

The extraction of RNA from the wheat seedlings was carried out using the phenolic method of Kirby (1968). The isoacceptor tRNAs were separated by RPC-5 column chromatography. The RPC-5 column was activated as described by Pearson et al. (1971), with a slight modification. Adogen 464 (20 ml) was used to activate 100 g Plascon CTFE 230. Elution took place using a 0.45–1.1 M L^{-1} linear NaCl gradient.

For the amino acylation, cytoplasmic aminoacyl–tRNA ligase was extracted from 8-day-old seedlings as described by Burkard et al. (1970) and Tamura et al. (1994). The labelling of tRNA was accomplished by incubation in 100 μL reaction mixture at 37°C for 20 min. The incubation solution contained 100 mM HEPES (pH 8.2), 10 mM $MgCl_2$, 1 mM ATP, 1 mM 2-mercaptoethanol, 8 μM ^3H amino acid (methionine, tyrosine or valine; specific acitivity 7.4×10^{10} Bq/mmol), 0.3 μg enzyme and 1 A_{260} unit tRNA.

Results

Studies were made on the effect of auxin, gibberellic acid and kinetin on the isoacceptor spectrum of tRNAs specific for the amino acids methionine, tyrosine and valine. The Σ tRNA pool was fractionated by RPC-5 column chromatography, and changes in the isoacceptor composition were monitored on the basis of changes in the chromatographic profile of tRNAs acylated using amino acids labelled with ^{14}C.

Changes in the $tRNA^{Met}$ isoacceptor spectrum as the result of hormone treatment

It can be seen from the RPC-5 chromatogram of wheat $tRNA^{Met}$, presented in Figure 1, that the acceptor tRNA of 4 different methionine-specific isoacceptors could be distinguished in untreated wheat seedlings (Fig. 1a), while five peaks were visible on the $tRNA^{Met}$ chromatogram of auxin-treated wheat (Fig. 1b). The new isoacceptor synthesised as the result of auxin was eluted at a concentration of 0.88 mol L^{-1} NaCl. A change in the isoacceptor ratio was also perceptible, peak 2 being the largest in auxin-treated wheat, while a shoulder appeared on peak 1.

Five methionine isoacceptors could be distinguished on the $tRNA^{Met}$ RPC-5 chromatogram of wheat treated with GA_3 (Fig. 1c). As the result of GA_3 treatment a new peak appeared at a concentration of 0.87 mol L^{-1} NaCl, and a shoulder, presumably indicative of a further isoacceptor not distinguishable with this method, on peak 2, which was again larger than in the untreated control.

The RPC-5 chromatogram of $tRNA^{Met}$ in wheat treated with kinetin (Fig. 1d) exhibited five distinct methionine isoacceptor tRNA peaks, of which peak 4, eluted at 0.92 mol L^{-1} NaCl, was absent in untreated plants. Like the other hormones, kinetin also caused an increase in the size of peak 2 and the appearance of a shoulder.

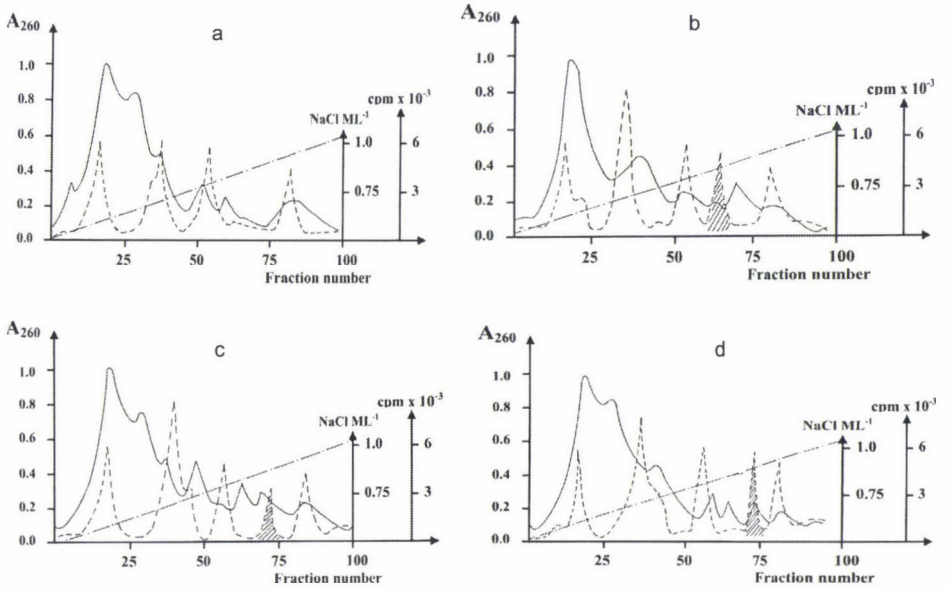

Fig. 1. Changes in the $tRNA^{Met}$ isoacceptor spectrum as the result of hormone treatment. — RPC-5 chromatographic profile of Σ tRNA, based on absorption at A_{260}. Elution was carried out using a 0.45–1.1 M L^{-1} linear NaCl gradient. – – – ^{14}C methionine activity, cpm × 10^3. a: untreated control; plants grown in liquid medium containing: b: 10^{-6} mol L^{-1} auxin, c: 10^{-6} mol L^{-1} gibberellic acid, d: 10^{-6} mol L^{-1} kinetin

Changes in the tRNA^{Tyr} isoacceptor spectrum as the result of hormone treatment

Figure 2 presents the RPC-5 chromatograms of wheat tRNA^{Tyr} and changes in the isoacceptor tRNA composition as the result of hormones. Five tyrosine acceptor peaks were detected on the untreated tRNA chromatogram (Fig. 2a), compared with six after treatment with auxin (Fig. 2b). The new peak appeared at an NaCl concentration of 0.90 mol L^{-1}. In addition to a change in the number of isoacceptors, the ratio of the various isoacceptors was also different in the auxin-treated variant: peaks 3 and 6 were larger than in the untreated plants, in addition to which the appearance of a new isoacceptor was observed as a shoulder on peak 6.

The tRNA^{Tyr} PCR-5 chromatogram of wheat treated with gibberellic acid (Fig. 2c) contained 7 peaks, indicating that two new tyrosine isoacceptor tRNAs appeared as the result of GA_3 treatment. These were eluted at NaCl concentrations of 0.68 and 0.90 mol L^{-1}. GA_3 also caused an increase in the size of peak 1 and a shoulder on peak 3, indicative of a further isoacceptor.

Seven tyrosine isoacceptor tRNAs could also be detected when wheat was treated with kinetin (Fig. 2d). The two new chromatographically distinguishable peaks were eluted at NaCl concentrations of 0.83 and 0.90 mol L^{-1}. A shoulder indicative of a further isoacceptor was observed on peak 6, while the size of peak 5 increased.

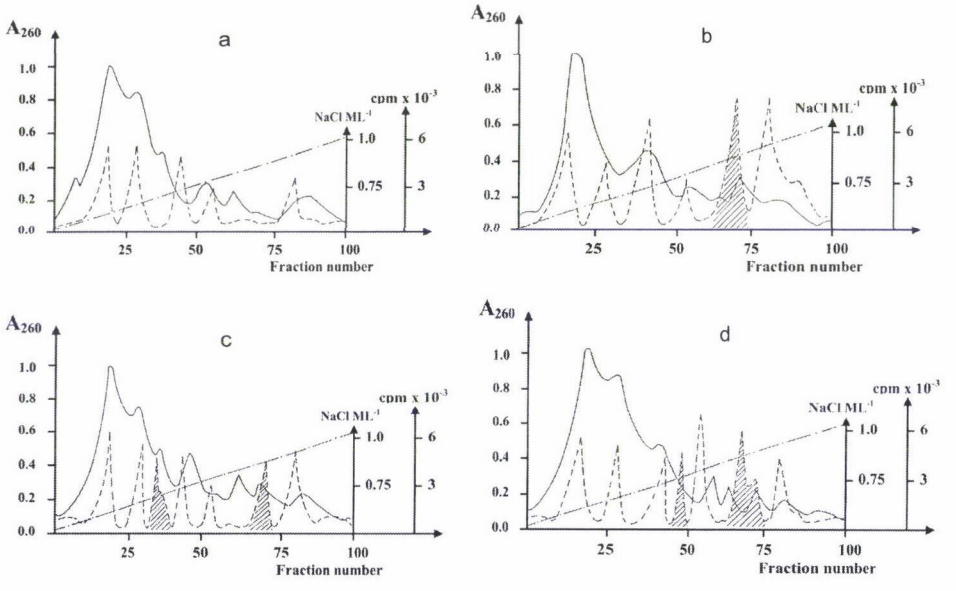

Fig. 2. Changes in the tRNA^{Tyr} isoacceptor spectrum as the result of hormone treatment. — RPC-5 chromatographic profile of Σ tRNA, based on absorption at A_{260}. Elution was carried out using a 0.45–1.1 M L^{-1} linear NaCl gradient. – – – ^{14}C methionine activity, cpm × 10^3. a: untreated control; plants grown in liquid medium containing: b: 10^{-6} mol L^{-1} auxin, c: 10^{-6} mol L^{-1} gibberellic acid, d: 10^{-6} mol L^{-1} kinetin

Changes in the $tRNA^{Val}$ isoacceptor spectrum as the result of hormone treatment

The $tRNA^{Val}$ RPC-5 chromatograms of wheat are presented in Figure 3. In untreated wheat nine valine isoacceptor peaks could be distinguished (Fig. 3a), while the auxin-treated variant contained ten peaks (Fig. 3b), with a new peak at an elution concentration of 0.90 mol L^{-1} NaCl. Differences were also observed in the sizes of the peaks. In untreated wheat the greatest acceptor activity was observed for peak 4, while after treatment with auxin peaks 2, 5 and 10 were larger than in the untreated control.

In wheat treated with GA_3 the $tRNA^{Val}$ RPC-5 chromatogram contained 11 valine isoacceptor peaks (Fig. 3), indicating the presence of two new isoacceptors, eluted at NaCl concentrations of 0.70 and 0.90 mol L^{-1}. In addition, peaks 1, 3, 6 and 10 increased in size.

Ten valine isoacceptor peaks could be distinguished on the $tRNA^{Val}$ RPC-5 chromatogram of kinetin-treated wheat (Fig. 3d). The new isoacceptor was eluted at a concentration of 0.60 mol L^{-1} NaCl. The acceptor activity of peaks 1, 7 and 9 was seen to increase.

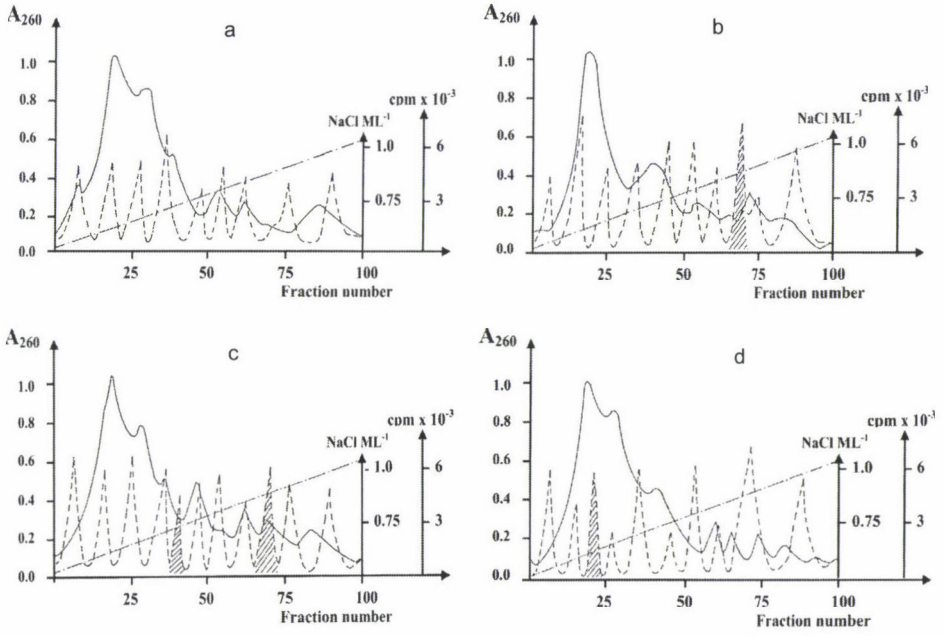

Fig. 3. Changes in the $tRNA^{Val}$ isoacceptor spectrum as the result of hormone treatment. — RPC-5 chromatographic profile of Σ tRNA, based on absorption at A_{260}. Elution was carried out using a 0.45–1.1 M L^{-1} linear NaCl gradient. – – – ^{14}C methionine activity, cpm × 10^3. a: untreated control; plants grown in liquid medium containing: b: 10^{-6} mol L^{-1} auxin, c: 10^{-6} mol L^{-1} gibberellic acid, d: 10^{-6} mol L^{-1} kinetin

Discussion

Little or no information is available in the literature on the effect of plant hormones on the tRNA metabolism or on the isoacceptor spectra of tRNAs. The present work was designed to fill this gap. Plant hormones play a decisive role in plant growth and development, inducing changes in gene expression, which then stimulate the synthesis of RNAs and proteins (Martin et al., 1996; Schmülling et al., 1997; Sitbon and Perrot-Rechenmann, 1997).

The results confirmed that plant hormones induced the expression of tRNA genes. For all three tRNAs exhibiting acceptor activity, treatment with any of the three plant hormones tested resulted in the appearance of one or two new isoacceptor tRNAs in wheat plants. Numerous papers have reported the stimulation of nucleic acid synthesis by plant hormones. It was demonstrated in bean hypocotyls that auxin stimulated RNA synthesis, leading to an increase in the RNA content of auxin-treated hypocotyls (Chen et al., 1983; Shingo et al., 1986). Kilev et al. (1983) and Gocal et al. (2001) reported that gibberellic acids stimulated both transcription and translation, and detected an increase in the mRNA and protein contents in pea epicotyls treated with gibberellic acid. Cytokinins are able to stimulate the RNA and protein synthesis of plant cells. Various authors (de la Serve et al., 1984; Jouanneau et al., 1984; Haberer and Kieber, 2002) found an increase in all the RNA fractions after cytokinin treatment.

The codon use of mRNAs stimulated by growth hormones differs from that of other mRNAs, so a special isoacceptor tRNA pool is required for protein synthesis (Lásztity et al., 1999; Osterman, 1977). This means that changes in the tRNA isoacceptor pool play an important role in the regulation of protein synthesis at the translation level (Hatfield and Rice, 1978; Osterman, 1977).

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Acta Agronomica Hungarica, 53(4), pp. 385–391 (2005)

SOURCES OF INFECTION AND METHODS OF CONTROL OF *SEPTORIA OENOTHERAE* IN EVENING PRIMROSE (*OENOTHERA* SPP.)

M. N. O'CONNELL[1], V. KETHEES WARARAJAH[2], A. F. FIELDSEND[1,3] and F. J. CULLUM[2]

[1]SCOTIA PHARMACEUTICALS LTD., PLANT TECHNOLOGY CENTRE, WRITTLE COLLEGE, CHELMSFORD, UK; [2]WRITTLE COLLEGE, CHELMSFORD, UK
[3]PRESENT ADDRESS: UNIVERSITY OF DEBRECEN CENTRE OF AGRICULTURAL SCIENCES, DEBRECEN, HUNGARY

Received: 23 May, 2005; accepted: 26 October, 2005

Septoria oenotherae West. can cause severe damage in overwintered crops of evening primrose (*Oenothera* spp.), including complete crop loss. Damage would be reduced if the sources of infection could be identified and removed. Examination of seed capsules inoculated with *S. oenotherae* showed that 96% of the pycnidia present were on the outside of the capsules, and seeds bearing pycnidia were only rarely found. However, internal infection of seeds from these capsules was demonstrated by both a blotter test and by culturing on agar media. Immersing seeds in 45°C water for 25 minutes destroyed viable fungal propagules located internally in seeds without reducing seed germination. The pathogen was also shown to overwinter in the pycnidial stage on stems left standing in the field. It is concluded that both internal seed-borne infection and overwintered crop debris are potential sources of infection in commercial seed stocks of evening primrose.

Key words: evening primrose, *Oenothera* spp., *Septoria oenotherae*, crop loss, seed-borne infection, hot water treatment

Introduction

Evening primrose (*Oenothera* spp.) is a high-value oilseed crop that is grown in temperate areas of northern and central Europe, North America and Australasia. It is a monocarpic biennial and the seeds are formed in capsules that are arranged in a spike. The seed oil is an important source of γ-linolenic acid, which has valuable nutritional and pharmaceutical properties (Horrobin, 1992). The crop may be either overwintered or spring-sown. Overwintered crops bolt more reliably, have fewer harvesting problems owing to their earlier maturity (Fieldsend and Morison, 2000a), and have higher seed oil contents (Fieldsend and Morison, 2000b), but are susceptible to damage by *Septoria oenotherae* West., which may result in complete crop loss (Simpson et al., 1995).

S. oenotherae was first reported to be seed-borne in evening primrose by Rawlinson and Dover (1986). Simpson et al. (1995) found that almost 75% of commercial seed stocks contained pycnidia of *S. oenothera* and that these were normally found on capsule, stem or leaf fragments and only rarely on the seeds. These pycnidia contained viable conidia and test seedlings of evening primrose became infected following inoculation with these conidia. Stobart et al. (1999) demonstrated a positive relationship between the number of pycnidia per gram of seed sown and the initial disease level in the field.

Separation of debris from infected seed stocks is difficult because evening primrose seeds are small (1 000 seeds weigh 0.4–0.5 g) and irregular in shape (Simpson et al., 1995). Furthermore the effectiveness of this control method would be reduced if *S. oenotherae* infection could be carried within the seeds. Stobart et al. (1999) observed *S. oenotherae* even in plots sown with seed free of pycnidia and suggested internal seed-borne infection as a source. This paper reports that internal *S. oenotherae* infection can be detected in evening primrose seeds and that hot water treatment is an effective method of control.

A further strategy for reducing the levels of *S. oenotherae* is to recommend a period of rotation between evening primrose crops. Simpson et al. (1995) have shown that the pathogen could survive when infected debris was buried in the soil for ten months. This paper demonstrates the potential of crop debris left standing in the field as a source of infection to subsequent crops.

Materials and methods

Plant material

In 1995, and again in 1996, 25 plants of an evening primrose breeding line known to be highly susceptible to *S. oenotherae* were raised under glass until the rosettes were approximately 100 mm in diameter, then planted out in April in a sheltered garden site in Writtle, Essex, UK. The plants were spaced 150 mm apart to encourage disease spread. Infected leaves collected from field crops were placed on the rosettes to inoculate them with *S. oenotherae*. Immediately after flowering an infected leaf was tied to the top of each flower spike to encourage capsule and seed infection. On dry days the plants were watered at dusk to facilitate disease spread. In October of both years capsules with high numbers of *S. oenotherae* pycnidia on their outer surfaces were individually harvested and allowed to dry.

Distribution of S. oenotherae *pycnidia on seeds and capsules*

The numbers of pycnidia on the lower and upper halves of 121 capsules harvested in 1995 were recorded using a stereo microscope (magnification ×20). Subsequently each capsule was opened and the number of pycnidia on the internal surface, the placenta and the seeds was counted. The percentage distribution of pycnidia was then calculated for each surface.

Assessment of internal infection in hand-harvested seeds

Ten heavily-infected capsules were harvested from the plants grown in 1996 and the distribution of pycnidia was again recorded. Seeds void of pycnidia were selected for assessment of internal infection based on a blotter test and an agar culture test as outlined for *S. nodorum* on *Triticum aestivum* (Anonymous, 1993). Forty seeds from each capsule were surface sterilised with 10% sodium hypochlorite for 3 min and then rinsed in sterile distilled water. For the blotter test, 90 mm diameter filter papers (Whatman 1, Merck Ltd., Dorset, UK) were set in the bottom and lid of a 100 mm Petri dish and wetted with 3 and 1 ml distilled water, respectively. Ten seeds were set in the prepared Petri dish, which was sealed with parafilm and incubated under fluorescent lights at 20°C for 21 days. The developing seedlings were checked for the presence of pycnidia at weekly intervals.

A further 40 seeds from each of the ten capsules were surface-sterilised, rinsed and transferred aseptically onto potato dextrose agar (PDA) (Merck Ltd., Dorset, UK). These Petri dishes were incubated as before and developing fungal colonies were recorded and identified over a 14-day period. Samples from colonies thought to be *S. oenotherae* were used to inoculate healthy evening primrose seedlings grown on cylindrical cellulose plugs as described by Simpson et al.

(1995). These were placed under fluorescent lights and the development of disease symptoms was recorded over 21 days. Larger pot-grown plants with rosette diameters of 200 mm and showing no disease symptoms were also inoculated. Agar from the leading edge of the colonies was placed on leaves at sites that had been pierced with a sterile needle to encourage infection. The plants were placed inside a large polyethylene bag and left on a north-facing window ledge for 21 days. After the completion of the PDA tests a dilute V8 agar medium, as used by Reeleder (1994) and an agar medium devised for the sporulation of *S. nodorum* (Richards, 1951) were used to culture *S. oenotherae* from infected evening primrose seeds.

Assessment of infection in combine-harvested seed stocks

A commercial seed crop of evening primrose cv. Rigel specially grown under a cropping regime designed to minimise *S. oenotherae* infection was harvested in October 1995. A 4 g sample of seeds was checked for the presence of pycnidia on seeds and debris. Following surface sterilising and rinsing, 400 seeds were plated onto PDA as described above. A further seed sample was harvested in autumn 1996 from untreated control plots in a fungicide trial. The incidence and severity of *S. oenotherae* in these plots was greater than would normally be expected in commercial crops (100% and 63%, respectively, six months prior to harvest). Agar and blotter tests were carried out using seeds from this sample.

Control of seed-borne S. oenotherae *using hot water treatment*

Four hundred pycnidia-free seeds from each of ten commercial crops of evening primrose cv. Rigel harvested in the Netherlands in 1997 and 1998 were tested for their germination (after 7 and 14 days) and *S. oenotherae* infection (after 21 days) percentages in April 2000 using the blotter test method previously described.

One of these seedlots was then used for the hot water treatment studies, using a method described by Bant and Storey (1952). For each temperature treatment, 400 seeds were enclosed in a muslin cloth and immersed in water maintained at the required temperature (ranging from 45°C to 58°C) for 25 minutes, then allowed to dry. After treatment the germination ability of the seeds and the level of infection with *S. oenotherae* were again measured using the blotter test method.

In all tests, 25 seeds were set per Petri dish, giving 16 Petri dishes per treatment.

Overwintering of S. oenotherae *on aerial plant debris*

The stems and remaining capsules of the inoculated test plants were left to overwinter and in April 1997 pycnidia containing conidia thought to be those of *S. oenotherae* were used to inoculate healthy evening primrose seedlings growing on cylindrical cellulose plugs. These were examined for signs of infection over a period of 21 days.

Results

Distribution of S. oenotherae *pycnidia on seeds and capsules*

Irregularly-shaped, fawn-coloured lesions of around 10 mm diameter containing developing pycnidia were commonly found on the rosettes of the inoculated plants. On flower spikes, disease symptoms were observed on many bracts and these subsequently spread to the capsules (Fig. 1). Lesions were also seen to start at the tip of the capsule. The spread of the disease was slow on immature green capsules but became more rapid as the capsules senesced. Lesions containing pycnidia also spread rapidly on the senescing stems with most of the stems being covered in pycnidia by November. Over 90% of pycnidia occurred on the outsides of the capsules with a significantly higher incidence ($T=2.46$, $p<0.05$) on the lower half of the capsule (Table 1). Only 0.4% were present on the seeds.

Internal infection by S. oenotherae *in hand-harvested seeds*

Similar data for the distribution of pycnidia were obtained in 1996 (Table 2). In the blotter test pycnidia only formed towards the end of the 21-day period as the seedlings were beginning to senesce. On PDA *S. oenotherae* recovered from seeds produced a dark colony with white edges which were 5–15 mm in diameter after 10–14 days. Pycnidia were malformed on PDA, based on the absence of an ostiole, and rarely contained distinct septate conidia. However, aseptic transfer of inoculum from these cultures onto healthy evening primrose seedlings or larger plants readily led to the development of typical disease symptoms that contained normal pycnidia within eight days. When plated on the medium developed for *S. nodorum*, a sparser, red-brown coloured colony grew from infected seeds. Again ostioles failed to form but septate conidia were found. Inoculation of healthy evening primrose seedlings again produced typical lesions of *S. oenotherae*. On dilute V8 agar, colonies similar in colour to those on PDA formed, with pycnidia again being malformed (Fig. 2). When *S. oenotherae* grew directly on seedlings germinating on the medium normal pycnidia were formed.

Infection by S. oenotherae *in combine-harvested seed stocks*

In the commercial seed sample no pycnidia were found on seeds or debris and no colonies of *S. oenotherae* were found on PDA (data not shown). Nineteen pieces of debris infected with pycnidia were found in the sample harvested from the fungicide trial control plots but no seeds bearing pycnidia were observed. After 21 days in the blotter test no seedlings infected with *S. oenotherae* were found and no colonies were formed on PDA.

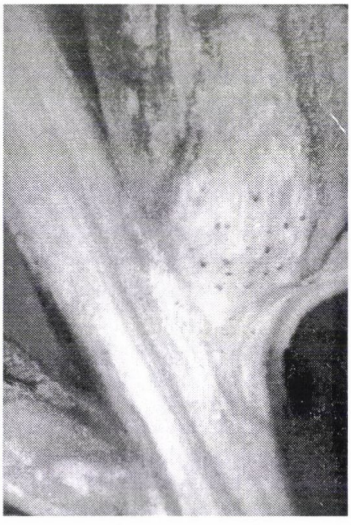

Fig. 1. Evening primrose (*Oenothera* spp.) bract with lesion and pycnidia associated with *S. oenotherae* infection that has spread to base of capsule

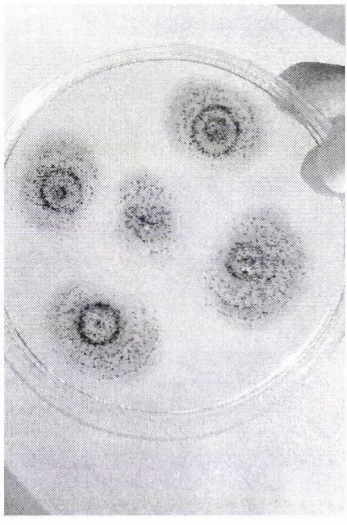

Fig. 2. Colonies of *S. oenotherae* developing from five evening primrose (*Oenothera* spp.) seeds after 14 days on dilute V8 agar. One seed has germinated and cotyledons are not yet showing symptoms of *S. oenotherae*

Table 1
Distribution of *S. oenotherae* pycnidia in 121 capsules of evening primrose (*Oenothera* spp.) harvested in 1995

Location	ESL	ESU	IS	Placenta	Seeds
Mean no. pycnidia per capsule	54.3	37.0	0.6	2.7	0.4
Standard error	5.3	4.6	0.2	1.0	0.2
% of total	57.2	39.0	0.6	2.8	0.4

ESL: External surface of capsule (lower half); ESU: External surface of capsule (upper half); IS: Internal surface of capsule

Table 2
Distribution of *S. oenotherae* in ten capsules of evening primrose (*Oenothera* spp.) harvested in 1996 and levels of internal seed infection

	No. of pycnidia on external capsule surface	No. of pycnidia on internal capsule surface	No. of pycnidia on placenta	No. of pycnidia on seeds	No. of infected seedlings/40 (Blotter Test)	No. of infected seeds/40 (PDA Test)
Mean	160.0	1.5	4.3	0	14.0	20.4
% of total	96.5	0.9	2.6	0		

Control of seed-borne S. oenotherae *using hot water treatment*

Using the blotter test *S. oenotherae* was detected in two (1997-harvested) seed stocks (Table 3) but not in the other eight (data not shown).

Seed stock 7 was used for the hot water treatment studies. The blotter test was repeated using 400 untreated seeds and similar data were obtained (Fig. 3). 87% of seeds had germinated after 14 days and three infected seedlings were detected after 21 days. 400 pycnidia-free seeds were then hot water treated at each of six temperatures and no pathogen infection was detected in any seed sample following hot water treatment.

In all treatments, few seeds germinated between 7 and 14 days and only the 14-day data are shown in Fig. 3. Seed germination declined steeply after immersion in water at temperatures greater than 50°C. A Kruskal-Wallis test confirmed that highly significant ($H=100$, $p<0.001$) differences in germination occurred across treatments, but a non-parametric Tukey-type multiple comparison test indicated that temperatures of 45 and 50°C caused no significant ($q=34.9$, $p<0.05$) reduction in germination.

Overwintering of S. oenotherae *on aerial plant debris*

Pycnidia containing abundant conidia typical of *S. oenotherae* could readily be found on overwintered dead stems and capsules. These conidia readily produced infection after 10–14 days when transferred to evening primrose seedlings growing on cylindrical cellulose plugs. Careful examination of this material over a number of weeks did not reveal a potential ascospore stage of this fungus.

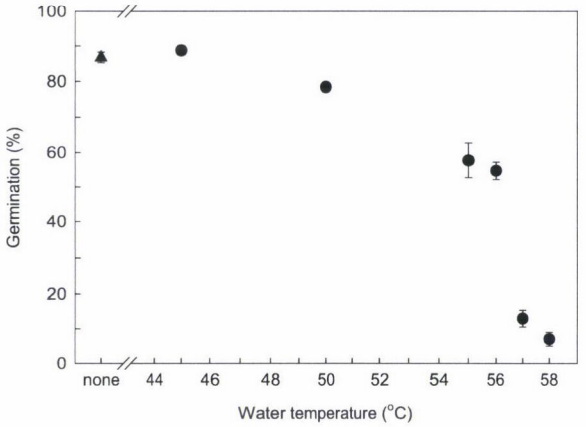

Fig. 3. Percentage seeds of evening primrose that had germinated after 14 days following soaking in hot water for 25 minutes at different temperatures (circles) and in the untreated control (triangle). Error bars represent ± 1 SE

Discussion

In the transplanted plants infection of capsules by *S. oenotherae* often followed infection of the subtending bracts and this may explain the higher number of pycnidia found on the lower half of the capsules. The number of pycnidia on the capsule surface increased markedly as the capsule ripened but few pycnidia were found on the internal capsule wall or on the placenta. Seeds bearing pycnidia accounted for only 0.4% of the pycnidia found on the 121 infected capsules examined (Table 1).

Internal infection by *S. oenotherae* was demonstrated not just in seeds exposed to an artificially high level of inoculum, but also, at a much lower level, in some commercial seed stocks (Tables 2 and 3). Although the incidence of infection in these stocks did not exceed 1%, at a sowing rate in commercial crops of approximately 800 seeds m^{-2} this represents an infection rate of up to 8 infected seeds m^{-2}. Stobart et al. (1999) have shown that even a low incidence of pycnidial infection (one seed per 2.5 m^2) can cause a significant increase in initial inoculum level. Thus, internal seed-borne infection cannot be ruled out as a potential primary source of infection in an otherwise healthy crop.

Hot water treatment at 45°C appears to be an effective method of controlling internal seed-borne infection by *S. oenotherae* in evening primrose. However, temperatures above 50°C caused a reduction in germination capacity (Fig. 1). A reduction in germination greater than 5–10% is considered unacceptable (Gratwick and Southey, 1986).

Table 3
Germination ability and levels of internal seed infection by *S. oenotherae* in seed stocks of evening primrose (*Oenothera* spp.) harvested in 1997

Seed stock No.	% germinated seed (7 days)	% germinated seed (14 days)	No. of infected seeds/400 (Blotter Test)
5	67	72	2
7	85	88	4

Strategies to reduce the incidence of *S. oenotherae* in commercial sowing stocks of evening primrose include the production of seed stocks in areas of low disease incidence (Simpson et al., 1995). In one such stock *S. oenotherae* could not be detected on either seeds or plant debris, nor was internal infection observed after plating the seeds onto agar.

Slow rosette growth during the winter months (Fieldsend and Morison, 2000a) means that crops are still susceptible to a low inoculum level if wet weather conditions prevail. The ease with which it has been shown that *S. oenotherae* can overwinter in aerial crop debris confirms that the agronomic practice of a rotation between evening primrose crops is a valuable precaution.

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Acta Agronomica Hungarica, 53(4), pp. 393–403 (2005)

N_2 FIXATION IN FABA BEAN GROWN UNDER SALINE AND NON-SALINE CONDITIONS USING A ^{15}N-TRACER TECHNIQUE

K. KHALIFA and F. KURDALI

AGRICULTURE DEPARTMENT, ATOMIC ENERGY COMMISSION, DAMASCUS, SYRIA

Received: 18 May, 2005; accepted: 7 November, 2005

A pot experiment was conducted to study the performance of faba bean and barley growing under saline conditions, in terms of dry matter yield, total N, and percentages and amount of N derived from the soil, fertilizer and atmosphere using a ^{15}N isotope dilution method. Three saline treatments were employed: 1) plants were grown on saline soil and irrigated with saline water (SsWs); 2) plants were grown on saline soil and irrigated with non-saline water (SsWn); and 3) plants were grown on non-saline soil and irrigated with saline water (SnWs). Furthermore, a control treatment was employed by growing the plants on non-saline soil and irrigating them with non-saline water (SnWn). The different salinity treatments reduced plant growth and the reduction was more pronounced in faba bean than in barley. However, the relative growth reduction of plants grown only on saline soil, using non-saline water, did not exceed 50% of the control, whereas the reduction exceeded 50% when plants were grown under completely saline conditions. The percentage of N_2 fixed (%Ndfa) was not affected negatively by saline conditions. The results clearly demonstrated that the effect of salinity on faba bean was more evident on plant growth than on N_2-fixing activity. Further studies are needed to obtain more salt-tolerant faba bean genotypes that possess a higher growth rate. This could simultaneously improve yield and N_2 fixation under severely saline conditions.

Key words: faba bean, barley, salinity, N_2 fixation

Introduction

Salinity is a widespread phenomenon restricting the growth of plants in many temperate regions, and large areas of agricultural land have gone out of production due to salt accumulation in their soils. Salinity generally inhibits plant growth through reduced water absorption, reduced metabolic activities due to Na^+ and Cl^- toxicity, and nutrient deficiency caused by ionic interference (Yao, 1983; Kurban et al., 1999).

Salt-affected lands in Syria, as in other parts of the world, are becoming needed for production in order to meet the food demands of the ever increasing human population and urbanization on earth. Problems of salinity may be addressed using technological and/or biological approaches (Fageria, 1992). Bio-saline agriculture could be a promising approach since it meets the above-mentioned demands (Qureshi and Barrett-Lennard, 1998).

It has been reported that plant species vary widely in their response to salt stress, ranging from extremely sensitive to tolerant species (Qureshi and Barrett-Lennard, 1998). Salt-tolerant leguminous plants are usually used to improve the

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fertility and productivity of salt-affected soils by adding substantial amounts of organic matter, increasing the availability of nutrients in the soil, and fixing atmospheric N_2 (Qureshi and Barrett-Lennard, 1998; Kurdali et al., 2003; Kurdali, 2004). Salinity has been reported to affect the plant growth of several legume species, such as faba bean (Yousef and Sprent, 1983; Zahran and Sprent, 1986; Cordovilla et al., 1996), soybean (Grattan and Maas, 1988) and chickpea (Elsheikh and Wood, 1990). Leguminous plants grown in highly saline environments require both free-living rhizobia and a salt-tolerant host (Craig et al., 1991; Cordovilla et al., 1996; Mashhady et al., 1998; Kurdali and Al-Ain, 2002).

Vicia faba L. (faba bean) is a source of high quality protein and is often grown on saline soils in the Middle East and Mediterranean regions. It is considered to be one of the most important grain legume crops in Syria. Faba bean is a moderately salt-tolerant plant species, with a yield decrease of 50% at soil electrical conductivity (EC) values ranging between 9 and 11 dS m^{-1} (Lockerman et al., 1983; El-Karouri, 1970). Other studies demonstrated that the dry matter production and nodulation in faba bean decreased when irrigated with saline effluent containing an EC of 10 dS m^{-1} (Ban-Uelos et al., 2002). However, the tolerance of faba bean, as well as of other plant species, to saline conditions depends on several factors, including the rhizobial strain, the concentration and nature of the salt, and the time of salinization (Babber et al., 2000; Zaghloul, 1981; Abdel-Ghaffar et al., 1982; Al-Tahir and Al-Abdulsalam, 1997).

Faba bean is grown in Syria on a large scale and is adapted to a variety of soil conditions, varying from moderately saline, to non-saline soil. Nevertheless, since large areas of the country suffer from salinity in the soil and/or irrigation water, the different levels of these saline conditions may affect the performance of this crop to varying degrees, particularly as regards N_2 fixation. In order to efficiently utilize salt-affected soil and water, it is important to determine the performance of crops under variable saline conditions. Therefore, this pot experiment was undertaken to study the performance of faba bean, grown on saline and non-saline soils and irrigated with either saline or non-saline water, in terms of dry matter yield, N uptake and N_2 fixation. The effect of saline conditions on the growth of barley, which was utilized as a reference crop for measuring the N_2 fixation by the legume crop, was also investigated.

Materials and methods

A pot experiment was conducted during the 2001 winter season at the research station of the Atomic Energy Commission of Syria at Deir-Alhajar, south east of Damascus, Syria. Each pot contained 8 kg of either saline or non-saline soil. Both soils were collected from different sites of the Euphrates valley in Syria, located about 20 km south east of Deir-Ezzor city. The main physical and chemical properties of the two experimental soils are given in Table 1.

Table 1
Main physical and chemical properties of the soils

Component	Saline soil	Non-saline soil
EC_e (dS/m)	13.84	0.90
pH	7.95	8.47
Org. matter (%)	0.18	1.94
$CaCO_3$ (%)	16.53	17.00
Ionic content (meq/l)		
Cl^-	82.00	2.62
HCO_3^-	2.68	2.30
CO_3^{2-}	–	–
Na^+	43.6	2.52
K^+	1.51	0.25
Ca^{2+}	7.17	1.00
Mg^{2+}	39.23	3.5
SO_4^-	6.83	2.35
CEC* (meq/100 g soil)	14.45	16.91
Na^+	0.14	0.46
K^+	0.06	0.59
Ca^{2+}	2.47	4.60
Mg^{2+}	9.47	8.73

* = Cation Exchange Capacity

Seeds of faba bean (*Vicia faba* L. cv. Hama 1) and barley (*Hordeum vulgare* L. cv. Arabi Abiad), as a non-fixing reference crop, were used. After germination, the seedlings were thinned to 4 and 10 plants per pot, for faba bean and barley, respectively.

For each of the two plant species investigated, a total of 16 pots, arranged in a randomized complete block design with four replicates, were used to test the following treatments:

SnWn: a control composed of plants grown on a non-saline soil (Sn) and irrigated with non-saline water (Wn).

SnWs: an experimental treatment composed of plants grown on non-saline soil (Sn) and irrigated with saline water (Ws).

SsWn: an experimental treatment composed of plants grown on saline soil (Ss) and irrigated with non-saline water (Wn).

SsWs: an experimental treatment composed of plants grown on saline soil (Ss) and irrigated with saline water (Ws).

The electrical conductivity values for saline soil, non-saline soil, saline irrigation water and non-saline irrigation water were 13.9 dS m^{-1}, 0.9 dS m^{-1}, 8.5 dS m^{-1} and 1.1 dS m^{-1}, respectively. The pots were set outdoors under natural climatic conditions.

A rate of ^{15}N-labelled ammonium sulphate (9.6337% ^{15}N atom excess) equivalent to 20 kg N/ha was applied at seed emergence.

The plants were harvested 15 weeks after planting. Shoot and root samples were dried at 70°C for 72 h, weighed and ground. Total N was determined by the Kjeldahl method (Bremner, 1965) and the $^{15}N/^{14}N$-isotope ratio was determined (IAEA, 1990) by emission spectrometry (Jasco-150, Japan). The N fraction derived from the atmosphere (%Ndfa) was calculated using the equation of Fried and Middelboe (1977).

The data were subjected to analysis of variance (ANOVA), and the means were compared using the least significant difference (LSD) test at the 0.05 level of probability ($P<0.05$).

Results

Dry matter yield and N uptake

Faba beans grown under non-saline conditions (SnWn treatment) produced significantly more dry matter yield than the other treatments (Table 2). The lowest value was obtained for plants grown under completely saline conditions (SsWs treatment). The dry matter yields of plants subjected to saline stress in the soil (SsWn) or in saline water (SnWs) did not significantly differ from each other. The reduction in dry matter yield of the aforementioned treatments did not exceed 50% of the control, indicating the possibility of growing faba beans under partially saline conditions.

The percentages and amounts of N uptake in different parts of faba bean are presented in Table 3. The relative reduction in N uptake by plants grown either in saline soil (SsWn) or with saline water (SnWs) did not exceed 50% of the control, whereas a significant negative effect was observed when plants were grown on saline soil and irrigated with saline water. These results indicated that the different salinity treatments reduced N uptake and that the reduction was more pronounced in faba beans grown under completely saline conditions than when grown under partially saline conditions.

Regarding barley plants, the pattern of salinity effects was relatively similar to that of faba bean. The dry matter yield of the shoots, roots and whole plant of barley in the SsWs treatment was significantly lower than that of the other treatments (Table 2). The highest dry matter yield in the whole plant was obtained in the SnWn treatment. The relative reduction in dry matter for plants grown either in saline soil (SsWn) or with saline water (SnWs) did not exceed 50% of the control, whereas it exceeded 50% when the plants were grown on saline soil and irrigated with saline water; however, this reduction was less pronounced in barley than in faba beans.

Table 2

Dry matter yield (g/pot) of faba bean and barley as affected by saline stress originating from soil and/or from irrigation water

Treatments	Dry matter yield (g/pot)		
	Shoots	Roots	Whole plant
Faba bean			
SnWn	12.75±0.32a	7.00±0.20a	19.75±0.48a
SnWs	08.13±0.77b	2.33±0.58b	10.45±1.28b
SsWn	07.88±0.63b	3.25±0.25b	11.13±0.59b
SsWs	02.35±0.20c	1.00±0.17c	03.35±0.27c
$LSD_{0.05}$	1.64	1.06	2.33
Barley			
SnWn	14.12±0.42a	4.26±0.07a	18.38±0.39a
SnWs	10.13±1.23b	2.18±0.08b	12.31±1.29b
SsWn	07.50±0.74c	2.03±0.09b	09.53±0.82c
SsWs	01.88±0.24d	0.70±0.02c	02.58±0.25d
$LSD_{0.05}$	2.34	0.22	2.64

SnWn: non-saline soil and non-saline water; SnWs: non-saline soil and saline water; SsWn: saline soil and non-saline water; SsWs: saline soil and saline water; Means within a column followed by the same letters are not significantly different (P>0.05)

Table 3
Nitrogen uptake in faba bean and barley plants as affected by saline stress originating from soil and/or from irrigation water

Treatments	%N		mg N/pot		Whole plant
	Shoots	Roots	Shoots	Roots	
Faba bean					
SnWn	3.18±0.14b	2.91±0.02b	404.1±14.2a	203.6±5.3a	607.8±19.2a
SnWs	3.50±0.32b	2.94±0.30ab	277.1±14.1b	063.1±8.1b	340.2±15.3b
SsWn	3.22±0.10b	2.48±0.11b	251.7±14.8b	080.6±7.7b	332.3±13.8b
SsWs	4.35±0.17a	3.61±0.31a	101.7±07.9c	036.5±7.7c	138.2±8.8c
$LSD_{0.05}$	0.61	0.69	40.2	22.5	45.4
Barley					
SnWn	1.55±0.07c	0.92±0.13b	217.5±4.3a	39.3±5.78a	256.8±7.2a
SnWs	2.05±0.20b	1.22±0.09b	199.9±4.9a	26.4±1.3b	226.3±3.8b
SsWn	2.22±0.13b	1.09±0.10b	164.0±7.6b	22.1±1.9bc	186.1±7.2c
SsWs	3.69±0.04a	1.92±0.07a	068.9±8.1c	13.5±0.76c	082.4±8.9d
$LSD_{0.05}$	0.39	0.31	19.95	9.68	21.60

SnWn: non-saline soil and non-saline water; SnWs: non-saline soil and saline water; SsWn: saline soil and non-saline water; SsWs: saline soil and saline water. Means within a column followed by the same letters are not significantly different (P>0.05)

The pattern of N uptake by barley was relatively similar to that of faba beans (Table 3). The different salinity treatments reduced N uptake and the reduction was more pronounced in barley grown under completely saline conditions than in that grown in the other treatments. The relative reduction in N uptake by plants grown either in saline soil (SsWn) or with saline water (SnWs) did not exceed 50% of the control, whereas it exceeded 50% when the plants were grown on saline soil and irrigated with saline water.

Nitrogen derived from the available sources

The percentage of N derived from the atmosphere (%Ndfa) in different plant parts of faba beans is shown in Table 4. In faba bean shoots these values were 56, 50, 56 and 68% in the SnWn, SnWs, SsWn and SsWs treatments, respectively, whereas they were 36, 44, 52 and 73% in the roots. The %Ndfa estimates for the whole plant were 50, 48, 55 and 69% for the aforementioned treatments, respectively. The total amounts of N_2 fixed in the whole plant were 302, 164, 181 and 97 mg N/pot in the SnWn, SnWs, SsWn and SsWs treatments, respectively. These results indicated that the effect of salinity was more evident on plant growth (dry matter yield and N uptake) than on %Ndfa.

The percentage of N derived from fertilizer (%Ndff) in faba bean was 3.62–5.46 in the shoots, 2.87–5.57 in the roots and 3.42–5.48% in the whole plant, while the amount of nitrogen derived from fertilizer (mg N/pot) ranged from 3.7–17.7 in the shoots, 0.98–9.7 in the roots and 4.7–27.4% in the whole plant. It was evident that the lowest percentages and amounts of Ndff in faba bean occurred when the plants were subjected to completely saline conditions.

The percentages and amounts of N derived from the soil (Ndfs) followed a similar trend to those of Ndff. These results indicated that the N uptake from soil and fertilizer was adversely affected by the high salinity level of the soil (e.g. SsWs treatment). However, the reduction in Ndff and Ndfs values in faba beans subjected to saline stress in the soil (SsWn) or in the irrigation water (SnWs) was less severe than in plants subjected to completely saline conditions.

Regardless of the salinity treatments, %Ndff and %Ndfs in the different plant parts of barley (Table 5) were higher than those in faba bean (Table 4). %Ndff in the whole plant of barley was significantly higher in the SsWs treatment than in the control (SnWn). However, the amounts of Ndff were considerably lower. The amounts of Ndfs in the different plant parts of barley behaved in a manner relatively similar to those of Ndff. The lowest value occurred in the SsWs treatment (73 mg N/pot) and the highest in the control (232 mg N/pot). On the other hand, Ndfs in plants grown only on saline soil (164 mg N/pot) was lower than in those grown on non-saline soil but irrigated with saline water (202 mg N/pot). This indicates that the adverse effect of saline stress on soil N uptake by barley was more pronounced when salts were present in the soil rather than in the irrigation water.

Table 4

Percentages (%) and amounts of N (mg N/pot) derived from atmosphere (Ndfa), fertilizer (Ndff) and soil (Ndfs) in faba bean plants as affected by saline stress originating from soil and/or from irrigation water

Treatments	Shoots		Roots		Whole plant	
	%	mg N/pot	%	mg N/pot	%	mg N/pot
Ndfa						
SnWn	56.3±2.2b	228.6±16.4a	35.9±1.4b	73.2±4.3a	49.5±1.9b	301.8±20.7a
SnWs	49.5±3.05b	136.5±07.4b	44.1±9.3b	27.13±5.7b	48.4±3.6b	163.6±8.3b
SsWn	55.5±4.6b	138.9±12.2b	51.9±2.5b	42.36±5.8b	54.5±3.9b	181.2±16.6b
SsWs	68.1±4.3a	069.2±06.7c	73.2±5.3a	27.31±7.2b	69.4±4.6a	096.5±11.4c
$LSD_{0.05}$	11.28	35.1	17.03	18.00	11.29	46.2
Ndff						
SnWn	4.4±0.2ab	17.7±0.3a	4.8±0.1b	9.8±0.2a	4.5±0.2ab	27.4±0.3a
SnWs	5.5±0.3a	15.2±1.4ab	5.6±0.9ab	3.6±0.9b	5.5±0.4a	18.8±1.9b
SsWn	5.4±0.6a	13.7±1.8b	5.3±0.3ab	4.2±0.2b	5.4±0.5a	17.9±1.7b
SsWs	3.6±0.5b	03.7±0.6c	2.9±0.6c	0.98±0.2c	3.4±0.5b	04.7±0.7c
$LSD_{0.05}$	1.29	3.65	1.73	1.47	1.27	3.98
Ndfs						
SnWn	39.3±2.0a	157.8±2.4a	59.3±1.3a	120.7±2.1a	46.0±1.8a	278.5±2.8a
SnWs	45.1±2.7a	125.5±11.7b	50.3±8.4ab	32.4±7.9b	46.1±3.2a	157.9±15.9b
SsWn	39.1±3.9a	99.2±12.9b	42.8±2.2b	34.1±1.9b	40.2±3.5a	133.3±11.8b
SsWs	28.3±3.8b	028.8±4.6c	23.9±4.7c	08.2±1.9c	27.1±4.1b	037.0±5.2c
$LSD_{0.05}$	9.99	28.04	15.30	13.31	10.03	31.80

SnWn: non-saline soil and non-saline water; SnWs: non-saline soil and saline water; SsWn: saline soil and non-saline water; SsWs: saline soil and saline water. Means within a column followed by the same letters are not significantly different (P>0.05)

Table 5
Percentages (%) and amounts of N (mg N/pot) derived from fertilizer (Ndff) and from soil (Ndfs) in barley plants as affected by saline stress originating from soil and/or from irrigation water

Treatments	Shoots		Roots		Whole plant	
	%	mg N/pot	%	mg N/pot	%	mg N/pot
Ndff						
SnWn	10.0±0.4c	21.8±1.0a	07.5±0.4b	2.9±0.5a	9.6±0.4c	24.7±0.9a
SnWs	10.8±0.3bc	21.5±0.6a	09.9±0.3a	2.6±0.2a	10.7±0.3bc	24.2±0.6a
SsWn	12.2±0.2a	20.0±0.9a	11.1±0.5a	2.4±0.1a	12.0±0.2a	22.4±0.9a
SsWs	11.3±0.5ab	07.7±0.8b	10.7±0.7a	1.4±0.1b	11.2±0.5ab	09.2±0.8b
$LSD_{0.05}$	1.06	2.66	1.50	0.78	1.12	2.51
Ndfs						
SnWn	90.0±0.4a	195.7±3.8a	92.6±0.4a	36.4±5.4a	90.4±0.4a	232.1±7.1a
SnWs	89.2±0.3a	178.3±4.7a	90.1±0.3b	23.8±1.2b	89.3±0.3ab	202.1±3.6b
SsWn	87.8±0.3b	144.0±6.2b	88.9±0.5b	19.7±1.8c	88.0±0.2c	163.7±6.4c
SsWs	88.7±0.5a	061.2±7.4c	89.3±0.7b	12.1±0.7c	88.8±0.9bc	073.3±8.1d
$LSD_{0.05}$	1.06	18.01	1.50	8.97	1.13	17.5

SnWn: non-saline soil and non-saline water; SnWs: non-saline soil and saline water; SsWn: saline soil and non-saline water; SsWs: saline soil and saline water; Means within a column followed by the same letters are not significantly different (P>0.05)

Discussion

Crops may be exposed, during their ontogeny, to a variety of environmental stresses. Salt stress is a principal factor that limits plant production, particularly when N nutrition depends on biological N_2 fixation (Abd-Alla, 1992; Abd-Alla et al., 2001).

In this study, the effect of saline stress originating from soil and/or from irrigation water on the performance of faba bean and barley was investigated. In faba bean, the different salinity treatments reduced dry matter yield, N uptake and the amount of N_2 fixed. The reduction was more pronounced in plants grown under completely saline conditions (>50%) than in plants exposed to either saline stress in the soil or saline stress in the irrigation water (<50%). Regarding barley plants, the pattern of salinity effects was relatively similar to that of faba bean. However, the reduction in barley growth was less pronounced than in faba bean, indicating that plant species vary in their response to salt stress. Moreover, the effect of saline water on barley dry matter yield was lower than that of saline soil. The estimated values of dry matter yield decrements induced by salinity stress in the water and soil were 33 and 48 % of the control, respectively.

Regarding N uptake, the pattern of salinity effects was relatively similar to dry matter yield. The percentage of N uptake decrements were 44, 45 and 77% of the control for faba bean plants subjected to saline water, saline soil, and both saline soil and saline water, respectively. In barley, the estimated values were considerably lower (12, 27 and 68% of the control for the afore-mentioned

treatments, respectively). The data obtained also indicated that the effect of saline stress originating from the soil on barley growth was more pronounced than that originating from irrigation water.

It has been reported that cereals are more efficient in soil N uptake than legume crops. This was clearly demonstrated when barley absorbed more nitrogen from the soil (Ndfs) than faba bean in all the treatments. Moreover, a number of studies have shown that salinity can reduce the uptake and utilization of mineral nutrients by plants (Abd-Alla et al., 2001; Grattan and Grieve, 1999; Marschner, 1995). Among the nutrients, N is one of the most widely limiting elements for crop production (Marschner, 1995). The amounts of N derived from the soil and from fertilizer in both plant species were substantially lower under saline conditions as compared to the non-saline conditions. The reduction in the percentage of soil N uptake was 13, 29 and 68% for barley, and 43, 52 and 87% for faba bean subjected to saline water, saline soil, and salinity in both soil and irrigation water, respectively. These results indicated that soil nitrogen uptake by plants was adversely affected by salinity, particularly when they were subjected to salinity in both soil and irrigation water. In addition, the severity of the adverse effect of salinity was more pronounced in faba bean than in barley, indicating that plant species differed greatly in their response to salinity.

In contrast to dry matter yield, N uptake and the amount of fixed N_2, the percentage of Ndfa in faba bean was not negatively affected by salinity. This points to differences in the effect of salt stress on the processes of plant growth and N_2 fixation in faba bean. As salinity severely affects plant growth, the total N_2 fixed, which is a yield-dependent parameter, may be a misleading indicator of the independent effect of stress factors on the N_2 fixation process *per se*. The parameter $\%N_2$ fixed is independent of dry matter production and could thus be useful for measuring the direct effect on N_2 fixation (Danso, 1986). Therefore, the successful symbiosis exhibited in the experimental treatments between faba bean and the local rhizobia in terms of N_2 fixation may be explained by the presence of effective salt-tolerant indigenous rhizobia strains. Giller and Wilson (1993) reported that there are marked differences between the adaptation of rhizobia strains to saline conditions and that the host legumes are generally much more sensitive to salinity than the bacteria. Some strains of rhizobia can actually grow in solutions with salinities as high as 43 dS m^{-1} (Singleton et al., 1982). This is perhaps not surprising in view of the fact that in the symbiotic state, rhizobia live within cells which have much greater solute concentrations than those generally experienced in soils (Giller and Wilson, 1993; Sprent, 1984). This would explain the data obtained in the present study, implying that the effect of salinity on faba bean was more evident on plant growth (dry matter yield and N uptake) than on %Ndfa, since the %Ndfa in plants grown under completely saline conditions was found to be higher than that in plants in the other treatments. Similarly, Abdel-Ghaffar (1988) reported that *Rhizobium leguminosarum* could tolerate a higher level of salinity than the host plant. This

reflects the importance of selecting cultivars of leguminous crops capable of withstanding salinity rather than attempting to introduce salt-tolerant rhizobial strains into the soil. Nevertheless, Soussi et al. (2001) demonstrated that the selection and characterization of salt-tolerant strains, which also show efficient symbiotic performance under salinity, may constitute a strategy for improving legume-rhizobia symbiosis in adverse environments. Therefore, further studies are needed to obtain more salt-tolerant faba bean genotypes that possess a higher growth rate and yield in order to improve their symbiotic performance with indigenous *R. leguminosarum* strains.

Conclusions

The overall data obtained on the effect of saline stress originating from soil and/or irrigation water on the performance of faba bean and barley showed that:

1. Both species were affected by salinity. However, they can be grown under partially saline conditions (saline soil or saline water stress) as the relative reduction in their dry matter yield and N uptake did not exceed 50% of the control.

2. The reduction in barley growth due to saline stress was less pronounced than in faba bean.

3. The effect of salinity on faba bean was more evident on plant growth than on N_2-fixing activity, reflecting the importance of selecting cultivars of faba bean capable of withstanding salinity rather than attempting to introduce salt-tolerant rhizobial strains into the soil.

Acknowledgements

We would like to thank Professor I. Othman, General Director of AECS, for his support. The technical assistance of the staff at the AECS Department of Agriculture is greatly appreciated.

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Acta Agronomica Hungarica, 53(4), pp. 405–415 (2005)

EFFECT OF N MANAGEMENT APPROACHES AND PLANTING DENSITIES ON NITROGEN ACCUMULATION BY TRANSPLANTED RICE

P. JANAKI and T. M. THIYAGARAJAN

DEPARTMENT OF SOIL SCIENCE AND AGRICULTURAL CHEMISTRY, TAMIL NADU AGRICULTURAL UNIVERSITY, COIMBATORE, TAMIL NADU, INDIA

Received: 30 September, 2004; accepted: 13 June, 2005

Field experiments were conducted during 1998 and 1999 in June–September with rice variety ASD18 at the wetland farm, Tamil Nadu Agricultural University, Coimbatore, India to find out the effect of N management approaches and planting densities on N accumulation by transplanted rice in a split plot design. The main plot consisted of three plant populations (33, 66 and 100 hills m^{-2}) and the sub-plot treatments of five N management approaches. The results revealed that the average N uptake in roots and aboveground biomass progressively increased with growth stages. The mean root and aboveground biomass N uptake were 26.1 to 130.6 and 6.4 to 17.8 kg ha^{-1}, respectively. The N uptake of grain and straw was higher in the *Sesbania rostrata* green manuring + 150 kg N treatment, but it was not effective in increasing the grain yield. The mean total N uptake was found to be significantly lower at 33 hills m^{-2} (76.9 kg ha^{-1}) and increased with an increase in planting density (100.9 and 117.2 kg ha^{-1} at 66 and 100 hills m^{-2} density). N application had a significant influence on N uptake and the time course of N uptake in all the SPAD-guided N approaches. A significant regression coefficient was observed between the crop N uptake and grain yield. The relationship between cumulative N uptake at the flowering stage and the grain yield was quadratic at all three densities. The N uptake rate (μ_N) was maximum during the active tillering to panicle initiation period and declined sharply after that. In general, μ_N increased with an increase in planting density and the increase was significant up to the panicle initiation to flowering period. Thereafter, the N uptake rate was similar at densities of 66 and 100 hills m^{-2}.

Key words: transplanted rice, N uptake, planting density, chlorophyll meter

Introduction

The knowledge of rice N requirements and uptake capacity is fundamental to the development of improved N management in rice. An important factor in fertilizer dose-response relationships is the rate at which the plant accumulates N and the timing of fertilizer applications to coincide with the rapid uptake period, leading to the more efficient use of fertilizer. The increase in N uptake with increasing N application levels has been well documented by several investigators (Thiyagarajan et al., 1991; Shi et al., 1994). The nitrogen uptake was found to increase rapidly under panicle initiation (PI), but thereafter tended to plateau and then decline as N and other nutrient elements were lost from the plants (Tanaka et al., 1964). As the amount of N absorbed to produce a unit grain yield is nearly constant, the plant must absorb a larger amount of N to produce higher grain yield (Murayama, 1979).

Data on the rate of N taken up by rice crops have been recorded recently as they were required for modelling purposes. Craswell and Godwin (1984) stated that the climatic factors that determine the growth rate of plants also determine the rate of N uptake from the soil. Stutterheim et al. (1994) reported the lack of any relationship between the rate of growth and N uptake, which was the result of different impacts of the environment on the two rates, e.g. an increase in temperature may decrease the growth rate through higher respiration, but it may increase the N uptake rate through higher root or mineralization activity. Peng and Cassman (1998) reported that at panicle initiation, a maximum N uptake rate of 9 to 12 kg ha^{-1} d^{-1} was measured over the 4-d period following the application of 100 kg N ha^{-1}, which was similar to the N uptake rate observed at mid-tillering. Therefore, this study was undertaken to find out the influence of different planting densities and N management approaches, specially the SPAD technique, on nitrogen (N) accumulation by transplanted rice.

Materials and methods

The two field experiments were conducted in 1998 and 1999 in June–September with rice variety ASD18 at the wetland farm of Tamil Nadu Agricultural University in Coimbatore, India. Coimbatore is situated in the Northwestern agroclimatic zone of Tamil Nadu at 11°N latitude and 77°E longitude and at an altitude of 426.7 m above mean sea level. The sowing and transplanting dates of the crop were the same in both years. The trials were located in two different fields of the same farm in 1998 and 1999, and the characteristics of the soils of the two fields are given in Table 1, as analysed by the procedure given by Jackson (1973).

Table 1
Characteristics of the soils (pre-puddling) of experimental sites at a ploughing depth of 0–15 cm

Soil characteristics	1998	1999
Clay (%)	46.2	41.8
Silt (%)	8.7	12.1
Sand (%)	45.1	46.1
pH	7.9	8.1
Electrical conductivity (d Sm^{-1})	0.4	0.5
Cation exchange capacity (cmol kg^{-1})	17.3	21.2
$KMnO_4$-N (kg ha^{-1}) – (Subbiah and Asija, 1956)	214	163
Olsen-P (kg ha^{-1}) – (Olsen et al., 1954)	16	13
NH_4OAc-K (kg ha^{-1}) – (Standford and English, 1949)	400	425
Total N (g kg^{-1})	5.22	3.86

A split-plot design with three replications was used. Three planting densities, namely 33 (30 × 10 cm); 66 (15 × 10 cm) and 100 (10 × 10 cm) hills m^{-2} CPD_{33}, PD_{66}, PD_{100} served as the main plots and five N regimes, namely Control (Minus-N); *Sesbania rostrata* green manure (SGM) @ 6.25 t ha^{-1} + 150 kg N ha^{-1} (SGM+N); SPAD guided N topdressing (SPAD); basal N @ 25 kg ha^{-1} + SPAD-N (Basal+SPAD); SGM @ 6.25 t ha^{-1} + SPAD-N (SGM+SPAD) as sub-plots. Fertilizer N was applied in four splits, i.e. 25% at 7 days after transplanting (DAT), 25% at the active tillering stage (21DAT), 25% at panicle initiation and the remaining 25% at 10 days after panicle initiation. In all the SPAD-N treatments, SPAD readings were taken at 7-day intervals

starting from 14 days after transplanting (14DAT) until first flowering, using the youngest fully expanded leaf of ten randomly selected plants from each plot. For SPAD management, N application as topdressing commences only from 14DAT if the observed chlorophyll meter value falls below the set threshold value. In existing recommended N application strategies, basal N application is recommended at a rate of up to 25–50% of the total N to be applied, besides 6.25 t ha^{-1} of green manure. Hence, to evaluate whether basal N is required for SPAD management, basal N through urea and SGM were also tried. The SPAD threshold value was set to 37. The quantity of N applied in different treatments is given in Table 2.

Table 2
Cumulative total N applied (kg ha^{-1}) in the different N management treatments and planting densities (hills m^{-2})

Planting density	SGM*+N		SPAD-N		Basal+SPAD		SGM*+SPAD	
	1998	1999	1998	1999	1998	1999	1998	1999
33	193	201	90	105	85	130	73	126
66	193	201	105	135	130	160	118	156
100	193	201	105	135	100	145	148	171

*Quantity of total N supplied through 6.25 t ha^{-1} (fresh weight) of green manure (*Sesbania rostrata*) was 43 kg in 1998 and 51 kg in 1999

The crop was harvested at physiological maturity and the grain yield from individual plots was recorded separately and expressed at 14% moisture content. The concentration of N in the grain sample was analysed by the Kjeldahl digestion method (using the required quantity of a digestion mixture of K_2SO_4 : $CuSO_4$: Se at a ratio of 1000:100:1, respectively and concentrated H_2SO_4, as described by Jones and Case, 1990) using a Kjelplus instrument for distillation. The titration was done manually using 0.02 N H_2SO_4. The uptake of nitrogen (N_u) was calculated by multiplying the biomass yield (kg ha^{-1}) by the corresponding nitrogen concentration of the plant parts at each growth stage. The sum of the N uptake of roots and aboveground biomass at the active tillering (AT), panicle initiation (PI) and flowering (FL) stages was expressed as the total N uptake (kg ha^{-1}) at each growth stage, and the sum of the uptake of straw, grains, unfilled spikelets and root at the harvest (HT) stage was expressed as total N uptake at HT. The N uptake rate (μ_N) was derived as follows (Thiyagarajan and Ten Berge, 1996):

The rate of N uptake between two consecutive growth stages (μ_N) was computed by dividing the incremental crop N uptake by the duration of the period between the relevant growth stages as shown in the following example and expressed as kg ha^{-1} d^{-1}.

$$\text{N uptake rate from AT to PI} = \frac{(\text{Crop } N_u \text{ at PI}) - (\text{Crop } N_u \text{ at AT})}{\text{Time interval between AT and PI (days)}}$$

The data obtained for both years was statistically analysed using the computer software IRRIStat (IRRI, 1993). The correlation and regression analysis were done as per the procedure given by Gomez and Gomez (1984).

Results and discussion

Nitrogen accumulation by plant parts

The amount of N present in crop biomass is termed as N accumulation or uptake. The N uptake in the root and aboveground biomass of rice seedlings at the time of transplanting is given in Table 3.

Table 3
Nitrogen uptake (kg ha^{-1}) in plant parts at the time of transplanting

Plant parts	33 hills m^{-2}		66 hills m^{-2}		100 hills m^{-2}	
	1998	1999	1998	1999	1998	1999
Aboveground biomass	0.50	0.59	1.02	1.21	1.55	1.82
Root biomass	0.16	0.16	0.33	0.33	0.49	0.49
Total	0.66	0.75	1.35	1.54	2.04	2.31

The average N uptake in roots and aboveground biomass at different growth stages (Tables 4 and 5) was significantly influenced by the different planting densities and N regimes. The mean root N_u and aboveground biomass N_u were only 8.9 and 69.2 kg ha^{-1} in 1999 as against the corresponding values of 15.8 and 92.8 kg ha^{-1} in 1998. The low N acquisition in 1999 may have been due to the poorer root growth as compared to 1998, which is confirmed by the more significant correlation between root biomass and total crop N uptake in 1998 (r^2=0.748) than in 1999 (r^2=0.577). Pons et al. (1989) reported that the acquisition of N by the plant involves costs in terms of carbon for the formation and maintenance of a root system as well as the ATP requirement for the uptake process itself. The nitrogen uptake of the grain and straw (Table 6) were also significantly higher in 1998 than in 1999. The N_u of the grain and straw was higher in the SGM+N treatment, but it was not effective in producing a significant increase in grain yield. All the three SPAD-guided N regimes led to similar N uptake and differed significantly from the SGM+N regime. The mean N_u of grain at PD_{33} was much lower than that at other densities. The relationship between total crop N uptake and grain yield at the flowering and harvesting stages is illustrated in Figure 1. The relationship between cumulative N_u at the flowering stage and the grain yield was quadratic in 1998 at all three densities. However, the relationship between N_u at the harvesting stage and the grain yield was different for different planting densities. The relationship was asymptotic for PD_{33} and PD_{66}, but quadratic for PD_{100}. The quadratic relationship observed in 1998 showed that N_u in 1998 was higher than necessary for the yield potential of the site. In 1999, the relationship between N_u at the flowering and harvesting stages and the grain yield was almost linear, except at the flowering stage at PD_{100}. For similar N_u, the grain yields were lower in 1999 than in 1998.

Significant differences were observed in the crop N_u at all the growth stages (Table 7). Total N uptake increased up to maturity in both the years, but the increase was significant up to the flowering stage. The major difference in the time course of mean N_u in 1998 and 1999 was the lower aboveground biomass N_u during the transplanting to active tillering and the panicle initiation to flowering periods in 1999. The lower total N_u in 1999 was probably associated with the poorer crop growth during the transplanting to active tillering period, which showed the lower native N supply at the early growth stage in 1999 when compared to 1998. The pattern of N uptake in Minus-N in both the years indicated a greater N supply in the active tillering to panicle initiation period.

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Table 4
Uptake of N (kg ha^{-1}) in aboveground biomass as influenced by different N regimes and planting densities

N regimes	33 hills m^{-2}			66 hills m^{-2}			100 hills m^{-2}			Mean		Net mean
	1998	1999	Mean	1998	1999	Mean	1998	1999	Mean	1998	1999	
Minus-N	50.8	29.6	40.2	61.5	33.4	47.5	70.9	40.9	55.9	61.1	34.6	47.8
SGM+N	89.1	70.6	79.8	114.8	86.1	100.4	134.5	89.5	112.0	112.8	82.1	97.4
SPAD	77.3	61.1	69.2	102.4	73.7	88.0	121.9	88.7	105.3	100.5	74.5	87.5
Basal+SPAD	70.2	61.9	66.1	110.6	80.4	95.5	122.4	91.7	107.0	101.1	78.0	89.5
SGM+SPAD	70.0	62.0	66.1	99.6	79.4	89.5	125.2	88.9	107.0	98.3	76.8	87.5
Mean	71.4	57.0		97.8	70.6		114.9	79.9				
Net mean			*64.3*			*84.2*			*97.5*	*94.8*	*69.2*	

$LSD_{5\%}$			
Year (Y)	2.31	Y×D	4.01
Planting density (D)	2.83	Y×N	4.42
N regimes (N)	3.13	D×N	5.42
		Y×D×N	NS

Table 5
Nitrogen uptake (kg ha^{-1}) in roots as influenced by different N regimes and planting densities

N regimes	33 hills m^{-2}			66 hills m^{-2}			100 hills m^{-2}			Mean		Net mean
	1998	1999	Mean	1998	1999	Mean	1998	1999	Mean	1998	1999	
Minus-N	7.3	3.3	5.3	11.2	4.6	7.9	14.4	6.2	10.3	10.9	4.7	7.8
SGM+N	11.7	8.9	10.3	18.8	10.6	14.7	28.0	10.6	19.3	19.5	10.0	14.8
SPAD	14.8	7.7	11.3	17.0	9.2	13.1	17.5	12.3	14.9	16.5	9.7	13.1
Basal+SPAD	11.7	7.5	9.6	20.0	10.5	15.3	18.8	12.9	15.9	16.8	10.3	13.6
SGM+SPAD	11.1	7.7	9.4	15.5	9.6	12.6	19.4	12.5	15.9	15.4	9.9	12.6
Mean	11.3	6.9		16.5	8.9		19.6	10.9				
Net mean			*9.2*			*12.7*			*15.3*	*15.8*	*8.9*	

$LSD_{5\%}$			
Year (Y)	0.98	Y×D	1.69
Planting density (D)	1.19	Y×N	1.36
N regimes (N)	0.96	D×N	1.67
		Y×D×N	2.36

Total N_u increased with an increase in planting density, which might be due to the higher biomass production of higher populations. The time course of N_u was similar for all the planting densities and the N_u at all the densities was significantly higher in 1998 than in 1999. N application had a significant influence on the N uptake and the time course of N uptake in all the SPAD-guided N approaches, regardless of whether basal N was applied through SGM or prilled urea. Higher N_u was observed for the SGM+N regime at all the growth stages at all the densities. This may be the result of the higher rate of N application. Increased N_u at higher N application levels has been reported by several investigators (Thiyagarajan et al., 1991; Shi et al., 1994).

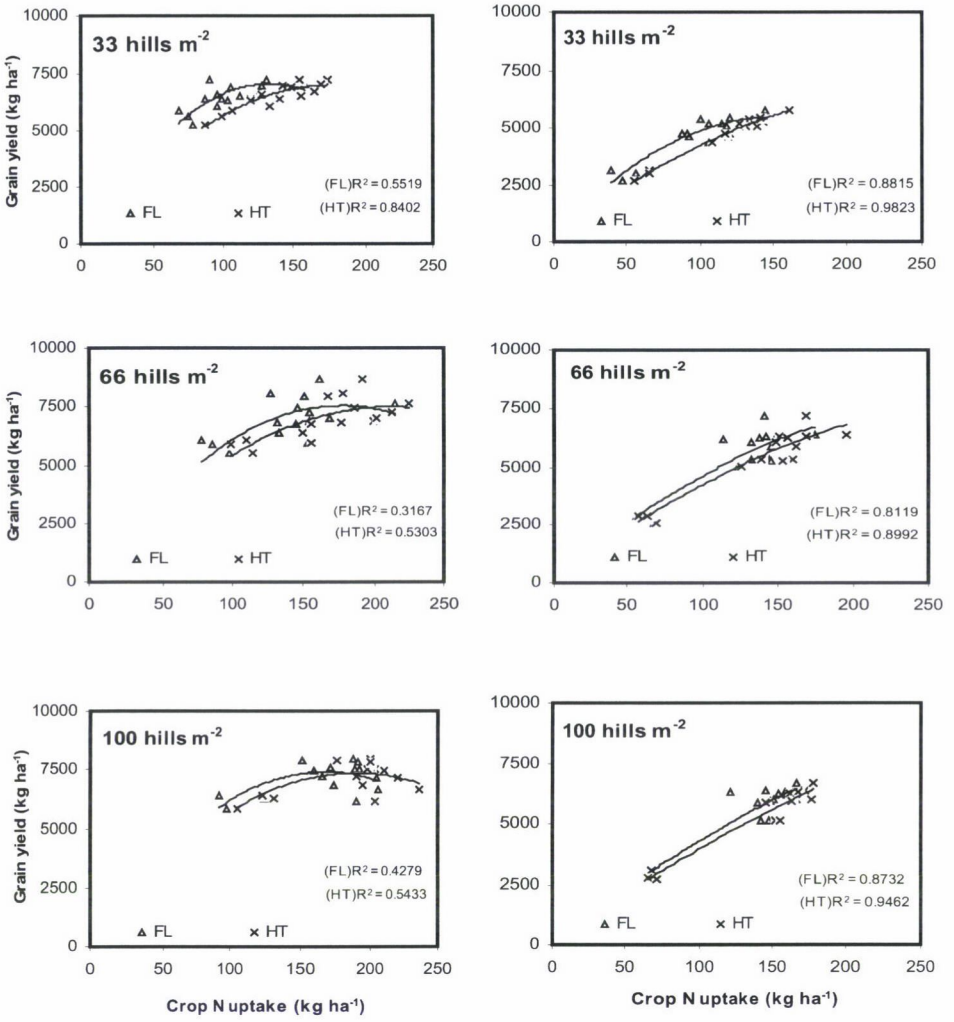

Fig. 1. Crop N uptake at flowering (FL) and harvesting (HT) stages versus grain yield at three planting densities in 1998 (left) and 1999 (right)

Maximum N_u was recorded in the SGM+N treatment at PD_{33} and PD_{66} in both years and at PD_{100} in 1998. In 1999, the N_u in SGM+N was very similar to that in other treatments where N was applied. The mean values for the different N regimes showed that all the SPAD-guided approaches were on par. The mean values under different N regimes at different planting densities ranged from 58.8 to 164.1 kg ha^{-1} in 1998 and 36.2 to 108.5 kg ha^{-1} in 1999. The cumulative N_u values at different growth stages in the three SPAD-N management systems were similar in spite of the fact that the time, source and quantity of N were different (Table 2). This clearly demonstrated the advantage of using the SPAD meter to control the N supply to the crop and facilitate a similar pattern of N_u by the crop.

Table 6
N uptake in grain, straw and unfilled spikelets (kg ha^{-1}) as influenced by different N regimes and planting densities

N regimes	33 hills m^{-2}			66 hills m^{-2}			100 hills m^{-2}			Mean	
	1998	1999	Mean	1998	1999	Mean	1998	1999	Mean	1998	1999
						Grain					
Minus-N	58.6	30.3	44.5	57.5	30.1	43.8	61.1	29.7	45.4	59.0	30.0
SGM+N	90.4	63.7	77.1	95.3	70.9	83.1	89.5	71.9	80.7	91.7	68.8
SPAD	79.6	57.4	68.5	86.8	64.5	75.7	92.3	66.2	79.2	86.2	62.7
Basal+SPAD	75.0	56.4	65.7	90.7	67.9	79.3	87.9	68.5	78.2	84.5	64.2
SGM+SPAD	69.7	57.2	63.5	87.5	60.5	73.9	98.9	60.0	79.5	85.4	59.2
Mean	74.6	53.0		83.5	58.8		85.9	59.3			
Net mean			*63.8*			*71.2*			*72.6*	*81.4*	*57.0*
						Straw					
Minus-N	27.6	11.0	19.3	36.0	11.6	23.8	40.3	14.6	27.5	34.6	12.4
SGM+N	57.2	27.7	42.4	82.0	32.9	57.4	83.8	40.3	62.0	74.3	33.6
SPAD	44.2	26.1	35.2	55.1	30.7	42.9	47.0	39.1	63.0	62.1	31.9
Basal+SPAD	44.8	21.0	32.9	64.4	28.4	46.4	72.9	29.7	51.3	60.7	26.3
SGM+SPAD	40.9	25.6	33.2	58.0	27.7	42.8	72.6	49.0	60.8	57.1	34.0
Mean	42.9	22.3		59.1	26.2		71.3	34.5			
Net mean			*32.6*			*42.7*			*52.9*	*57.8*	*27.7*
						Unfilled spikelets					
Minus-N	1.6	5.6	3.6	2.2	2.6	2.4	2.2	4.7	3.5	1.9	4.3
SGM+N	4.4	7.5	5.9	6.2	10.1	8.1	6.3	9.1	7.7	5.6	8.9
SPAD	5.1	7.2	6.2	2.9	8.8	5.9	5.1	6.1	5.6	4.4	7.4
Basal+SPAD	2.6	5.2	3.9	5.1	6.3	5.7	6.7	8.0	7.4	4.8	6.5
SGM+SPAD	2.8	11.8	7.3	4.2	13.1	8.6	4.8	6.2	5.5	3.9	10.4
Mean	3.3	7.5		4.1	8.2		5.0	6.8			
Net mean			*5.4*			*6.2*			*5.9*	*4.1*	*7.5*

$LSD_{5\%}$ Source	Grain	Straw	Unfilled spikelets
Year (Y)	4.78	3.42	0.86
Planting Density (D)	5.85	4.19	NS
N regimes (N)	4.64	4.73	1.42
Y×D	Not significant (NS)	5.92	NS
Y×N	NS	6.69	2.01
D×N	NS	8.19	2.46
Y×D×N	NS	NS	NS

N uptake rate

The N uptake rate (μ_N) of the crops, observed during the different growth periods, is presented in Table 8. The mean values of μ_N during the different growth periods in both years showed that the maximum μ_N occurred from transplanting to active tillering in 1998 and that the rate decreased gradually with growth. In 1999, however, the μ_N from transplanting to active tillering was very low compared to 1998 and the peak μ_N was observed during the active tillering to panicle initiation period, after which it declined sharply. The mean μ_N values were very similar from panicle initiation to flowering in both years. These results may also be a reflection of the native fertility of the soils or the inherent soil constraints

Table 7
Total N uptake (kg ha^{-1}) at different growth stages as influenced by different N regimes and planting densities (hills m^{-2})

Planting density	Active tillering			Panicle initiation			Flowering			Harvesting		
	1998	1999	Mean	1998	1999	Mean	1998	1999	Mean	1998	1999	Mean
Minus-N												
33	19.3	5.0	12.1	44.9	28.3	36.6	73.0	48.6	60.8	97.8	62.9	80.4
66	38.2	10.7	24.4	58.6	31.4	45.0	87.1	62.2	74.6	108.6	63.2	85.9
100	52.7	14.4	33.6	65.4	52.0	58.7	104.9	67.9	86.4	120.3	68.6	94.5
Mean	36.7	10.0		56.3	37.2		88.3	59.6		108.9	64.9	
Net mean			*23.4*			*46.7*			*73.9*			*86.9*
SGM+N												
33	24.3	7.3	15.8	76.4	57.3	66.8	138.8	136.2	137.5	169.2	147.9	158.6
66	50.1	14.9	32.5	103.3	79.6	91.5	178.8	152.6	165.7	208.3	171.3	189.8
100	99.5	23.4	61.5	148.6	110.1	129.4	194.3	136.6	165.4	213.8	161.0	188.8
Mean	58.0	15.2		109.4	82.3		170.6	141.8		197.1	163.8	
Net mean			*36.6*			*95.8*			*156.2*			*179.0*
SPAD												
33	26.5	8.0	17.2	82.8	64.4	73.6	115.6	98.6	107.1	149.0	125.4	137.2
66	50.6	13.7	32.2	112.2	69.4	90.8	153.0	129.9	141.5	167.0	148.5	157.8
100	67.4	21.3	44.3	112.6	107.4	110.0	176.0	151.2	163.6	206.4	163.3	184.9
Mean	48.1	14.3		102.5	80.4		148.2	126.6		174.1	145.8	
Net mean			*31.2*			*91.5*			*137.4*			*159.9*
Basal+SPAD												
33	30.0	12.0	21.0	71.3	70.6	70.9	92.2	104.3	98.2	138.3	118.9	128.6
66	51.2	21.0	36.1	140.9	84.4	112.7	150.4	139.0	144.7	188.0	153.3	170.7
100	88.4	25.6	56.9	125.0	105.9	115.5	165.1	156.9	160.9	189.5	168.4	178.9
Mean	56.5	19.5		112.4	86.9		135.9	133.4		171.9	146.4	
Net mean			*38.0*			*99.7*			*134.6*			*159.4*
SGM+SPAD												
33	26.1	8.6	17.4	75.8	55.0	65.4	98.3	108.3	103.3	127.8	130.9	129.4
66	54.9	14.2	34.6	109.6	73.5	91.6	131.1	141.4	136.3	167.3	155.7	161.4
100	73.0	21.8	47.4	120.0	100.9	110.5	189.8	155.8	172.8	198.5	161.9	180.2
Mean	51.3	14.9		101.8	76.5		139.7	135.2		164.5	149.5	
Net mean			*33.1*			*89.1*			*137.5*			*157.0*
Stage mean	50.1	14.8	32.4	96.5	72.6	84.6	136.5	119.3	127.9	163.3	133.6	148.5

$LSD_{5\%}$						
Year (Y)	2.92	Y×D	5.05	Y×S×D	10.10	
Growth stage (S)	4.12	Y×N	4.75	Y×S×N	9.49	
Planting density (D)	3.57	S×D	7.14	Y×D×N	8.22	
N regimes (N)	3.36	S×N	6.93	S×D×N	12.01	
Y×S	5.83	D×N	6.00	Y×S×D×N	Not significant	

limiting the activity of the roots in the 1999 field, especially during the establishment period of the crop after transplanting (Thiyagarajan and Ten Berge, 1996). The mean N uptake rate ranged from 0.34 to 4.65 kg ha^{-1} d^{-1} in 1998 and 0.02 to 4.13 kg ha^{-1} d^{-1} in 1999. In general, μ_N increased with an increase in planting density. The increase was significant up to the panicle initiation to flowering period; thereafter, μ_N was similar at PD_{66} and PD_{100}. There was significantly higher μ_N in PD_{33} during the flowering to harvesting period, which

might be one of the reasons for the yield potential at this density. The mean μ_N values at different planting densities (Table 8) varied significantly from each other (1.38 to 1.84 kg $ha^{-1}d^{-1}$). Stutterheim et al. (1994) reported that the initial plant population density had a great influence on the dynamics of shoot N uptake rates.

Table 8
Nitrogen uptake rate (kg ha^{-1} d^{-1}) during different growth periods as influenced by different N regimes and planting densities (hills m^{-2})

Planting density	TP–AT			AT–PI			PI–FL			FL–HT		
	1998	1999	Mean	1998	1999	Mean	1998	1999	Mean	1998	1999	Mean
Minus-N												
33	0.88	0.20	0.54	1.23	1.11	1.17	1.17	0.81	0.99	0.95	0.59	0.77
66	1.75	0.43	1.09	0.97	0.99	0.98	1.19	1.23	1.21	0.82	0.04	0.43
100	2.41	0.58	1.49	0.61	1.79	1.19	1.65	0.63	1.14	0.60	0.02	0.31
Mean	1.68	0.40		0.93	1.29		1.34	0.89		0.79	0.22	
Net mean			*1.04*			*1.12*			*1.11*			*0.51*
SGM+N												
33	1.13	0.32	0.72	2.48	2.38	2.42	2.61	2.82	2.71	1.17	0.51	0.84
66	2.32	0.64	1.48	2.54	3.08	2.81	3.15	2.61	2.88	1.14	0.82	0.98
100	4.65	1.00	2.83	2.34	4.13	3.23	1.91	0.95	1.43	0.75	1.19	0.97
Mean	2.69	0.65		2.46	3.19		2.55	2.12		1.02	0.84	
Net mean			*1.68*			*2.82*			*2.34*			*0.93*
SPAD												
33	1.23	0.34	0.79	2.68	2.69	2.68	1.37	1.37	1.37	1.28	1.12	1.20
66	2.34	0.58	1.46	2.94	2.65	2.79	1.71	2.42	2.06	0.54	0.77	0.66
100	3.11	0.90	2.01	2.16	4.10	3.13	2.65	1.76	2.20	1.17	0.50	0.83
Mean	2.23	0.61		2.59	3.14		1.91	1.85		1.00	0.79	
Net mean			*1.42*			*2.87*			*1.88*			*0.89*
Basal+SPAD												
33	1.39	0.54	0.97	1.97	2.79	2.68	0.88	1.35	1.11	1.78	0.61	1.19
66	2.37	0.93	1.65	4.27	3.02	3.65	0.41	2.18	1.29	1.45	0.59	1.02
100	4.11	1.11	2.61	1.75	3.82	2.78	1.67	2.04	1.85	0.94	0.48	0.71
Mean	2.63	0.86		2.66	3.21		0.99	1.86		1.39	0.56	
Net mean			*1.74*			*2.94*			*1.42*			*0.97*
SGM+SPAD												
33	1.21	0.37	0.79	2.36	2.21	2.28	0.95	2.13	1.54	1.13	0.94	1.04
66	2.55	0.65	1.59	2.61	2.77	2.69	0.90	2.72	1.81	1.40	0.59	0.99
100	3.38	0.93	2.16	2.24	3.77	3.00	2.92	2.19	2.55	0.34	0.25	0.29
Mean	2.38	0.65		2.40	2.92		1.59	2.35		0.96	0.59	
Net mean			*1.52*			*2.66*			*1.97*			*0.77*
Period mean	2.32	0.63	***1.48***	2.20	2.75	***2.48***	1.66	1.81	***1.74***	1.02	0.60	***0.82***

$LSD_{5\%}$						
Year (Y)	0.113	Y×D	0.192	Y×P ×D	0.391	
Growth period (P)	0.159	Y×N	NS	Y×P×N	0.456	
Planting density (D)	0.180	P×D	0.277	Y×D×N	NS	
N regimes (N)	0.161	P×N	0.465	S×D×N	0.577	
Y×P	0.226	D×N	NS	Y×P×D×N	0.816	

TP: transplanting; AT: active tillering; PI: panicle initiation; FL: flowering; HT: harvesting; NS: not significant

The effect of N regimes on μ_N was significant and the trend of μ_N was similar under all treatments where N was applied during the transplanting to active tillering and the active tillering to panicle initiation periods, in spite of the fact that the quantity of N applied was different in different N regimes. The mean μ_N for the N treatments showed large variations during the panicle initiation to flowering period. The μ_N from flowering to harvesting was 0.59 kg ha^{-1} d^{-1} for PD_{33} and 0.04 and 0.02 kg ha^{-1} d^{-1} for PD_{66} and PD_{100}, respectively (Table 6), indicating that at higher planting density N_u was lower during the flowering to harvesting period, too. The maximum μ_N for all the N regimes at PD_{100} occurred during the transplanting to active tillering period in 1998 and during the active tillering to flowering period in 1999. The highest value recorded was 4.63 kg ha^{-1} in 1998 and 4.13 kg ha^{-1} in 1999.

It can be concluded from this study that the total N uptake increased with an increase in planting density due to the higher biomass production of higher populations. The higher N uptake of the grain and straw in the SGM+N treatment was not effective in producing a significant increase in grain yield. The cumulative N uptake at different growth stages in the three SPAD-N management systems was similar in spite of the fact that the time, source and quantity of N were different. The N uptake rate was maximum during the transplanting to active tillering stage and increased with an increase in planting density. These results confirm that the planting population and different nitrogen management practices have a definite influence on the N uptake and grain yield production of the rice crop.

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Acta Agronomica Hungarica, 53(4), pp. 417–422 (2005)

EFFECT OF DIFFERENT PLANTING DENSITIES ON YIELD AND YIELD COMPONENTS OF WILD THYME (*THYMBRA SPICATA* VAR. *SPICATA*)

S. KIZIL and Ö. TONÇER

DEPARTMENT OF FIELD CROPS, FACULTY OF AGRICULTURE, DICLE UNIVERSITY, DIYARBAKIR, TURKEY

Received: 1 February, 2005; accepted: 10 November, 2005

The study reports the effect of the planting density of wild thyme (*Thymbra spicata* var. *spicata*) on plant height, fresh herbage yield, dry herbage yield, dry leaf yield, essential oil content and essential oil yield. The results show that the highest fresh and dry herbage, dry leaf yield and essential oil yield were obtained from narrow row spacing and in-rows. However, fresh herbage, dry leaf yield and essential oil yield showed a variation of 0.993–1.463 kg m^{-2}, 0.179–0.279 kg m^{-2} and 33.6–50.9 L ha^{-1}, respectively.

Key words: *Thymbra spicata* var. *spicata*, plant density, dry leaf and essential oil yield

Introduction

"Kekik" is a collective vernacular name given to species which smell like thyme and contain thymol and carvacrol in their essential oils. Several genera in the *Labiatae* have this scent, notably *Origanum, Thymus, Thymbra, Satureja* and *Coridothymus* (Başer, 1995; Özhatay and Atay, 1997).

Thyme (*Thymbra spicata* var. *spicata*), which is a medicinal plant, is considered to have anthelmintic, antispasmodic, carminative, emmenagogue, expectorant, rubefactient, sedative, stimulant and tonic characteristics. Thyme has also been used to promote perspiration (Simon et al., 1984). *T. spicata* var. *spicata* is commonly found in Southeast Anatolia, Turkey, as a perennial subshrub (Davis, 1982). Dried herbal parts of *Thymbra* are used as a herbal tea, condiment and folk medicine to treat asthma, colic, bronchitis, coughs, diarrhea and rheumatism in Turkey. Other uses include the flavouring of cheese, soup, toothpaste, mouthwashes, cough syrups, stew, stuffing, meat, fish, dressings, sauces, perfumes, cosmetics and honey (Baytop, 1984; Simon et al., 1984). The main essential oil component of *T. spicata* is carvacrol (39–77%) (Başer, 2002), while the other components are p-cymene, gamma-terpinene and thymol, a powerful antiseptic (Akgül and Özcan, 1999). *Thymbra* essential oil is composed of 53.1% oxygenated compounds, 25.7% monoterpene hydrocarbons and 4.4% sesquiterpenes (Hanci et al., 2003).

Very little attention has been given to the production or utilization of thyme, even though it is used by a large variety of people in folk medicine. This makes it seem desirable that studies should be carried out to achieve higher yield. Agricultural practices such as spacing and harvesting have a critical effect on the quantitative and qualitative characteristics of thyme, which finally result in plant growth and yield increment (Badi et al., 2004). Agronomical studies on *T. spicata* are also very limited. Therefore, the aim of the present study was to determine the yield and yield components of *T. spicata* var. *spicata* at different plant densities.

0238–0161/$ 20.00

Materials and methods

The experiment was conducted at the Department of Field Crops, Faculty of Agriculture, Dicle University, Diyarbakır (latitude 37°53′N and longitude 40°16′E, 680 m above sea level) during 2001, 2002 and 2003 on seeds collected from wild plants. Meteorological data are given in Table 1.

Seeds of *T. spicata* var. *spicata* were sown in a seedbed nursery, then transplanted to the field when they reached a plant height of 10–15 cm (April 2001). The field trial was laid out in a randomised block design with three replications. Each plot was arranged as 4 rows each of 30×20, 30×30, 30×40, 40×20, 40×30, 40×40, 50×20, 50×30, 50×40, 60×20, 60×30 and 60×40 cm, with a plot size of 3.6 m^2 (1.2 m × 3 m) for 30 cm row spacing, 4.8 m^2 (1.6 m × 3 m) for 40 cm, 6 m^2 (2 m × 3 m) for 50 cm and 7.2 m^2 (2.4 m × 3 m) for 60 cm. During the vegetation period, the plots were irrigated and weeded as and when required. No harvesting was done during the first year, while the second and third year harvests took place on June 8, 2002 and June 20, 2003, at the flowering stage, to evaluate the effects of plant density on plant height, fresh herbage yield, dry herbage yield, dry leaf yield, essential oil content and essential oil yield.

Omitting the border rows, the plants were cut at a height of 10 cm above the soil and weighed to determine fresh herbage yield, while the dry herbage yield was determined by drying fresh herbage samples from each plot in a shaded, airy place for a week. Dry leaf yields were determined after separating the leaves and stems of the dry herbage samples. Essential oil content was measured volumetrically, by hydrodistillation using a Clevenger apparatus, on 20 g samples taken from each plot (%, v/w). The essential oil content of each plot was multiplied by dry leaf yield to determine the essential oil yield (L ha^{-1}).

The data were analysed statistically using the MSTAT-C computer program (Michigan State University, USA), and the means were grouped using LSD values at a significance level of 5%.

It is evident (Table 1) that mean precipitation during the first and third years was higher than the many years' mean, whereas the mean precipitation during the second year was lower than the mean. During the vegetation period (March–June) the mean temperature was close to the long-term mean and the mean humidity of each year was lower than the mean over many years.

Table 1

Temperature (°C), humidity (%) and precipitation (mm) means at the experimental site over many years and in 2001–2003

Months	Many years			2001			2002			2003		
	Temp.	Hum.	Precip.	Temp.	Hum.	Precip.	Temp.	Hum.	Precip.	Temp.	Hum.	Precip.
January	1.6	76	73.5	4.0	67	14.9	0.7	77	31.2	4.0	78	68.4
February	3.6	72	67.1	5.0	66	72.4	5.6	58	46.1	2.5	76	151.8
March	8.1	65	67.9	11.4	69	126.1	9.4	64	73.0	6.5	64	80.7
April	13.8	63	70.5	14.3	64	54.0	12.2	69	65.0	13.4	66	80.6
May	19.3	56	42.1	16.7	60	86.9	17.9	48	34.9	20.4	45	5.4
June	25.9	37	7.0	26.7	26	0.0	26.3	29	1.3	26.4	25	26.9
July	31.0	27	0.7	31.6	22	0.0	31.0	19	0.0	31.7	14	0.0
August	30.3	27	0.5	30.2	25	0.0	29.8	22	0.0	31.5	15	0.3
September	24.8	32	2.7	24.7	27	0.0	25.0	27	5.5	25.0	21	0.9
October	17.0	48	31.1	16.3	51	67.0	18.6	41.9	15.7	19.0	40	33.3
November	9.6	67	54.0	7.0	61	52.3	10.2	55.3	36.6	9.0	68	62.5
December	4.1	67	71.5	5.1	82	131.7	4.0	71	74.4	4.0	76	87.9
Total			490	193		605.5	190.7		383.6	193.4		598.7

Source: Diyarbakır Meteorology Bulletin (2004)

Results and discussion

The results (Tables 2 and 3) showed wide variations due to plant densities in all investigated characters, except the essential oil content from year to year, with higher yield in 2003.

In the cultivation of all types of thyme, it is important to keep high plant length, which affects the yield of herbage. The plant density did not significantly affect the plant height; however, the varying meteorological conditions in the different years had a significant ($P<0.05$) effect on the plant height, so that the plants were taller in 2003 than in 2002. The data on plant height are lower than those reported by Tansı (1991), primarily due to the differing ecological conditions under which the experiments were conducted, but compatible with the findings of Kızıl and Tonçer (2003), because of the similar experimental conditions.

Sometimes *T. spicata* herbage is used as green material, so a high fresh herbage yield is desirable to achieve high dry herbage yield. The sowing space significantly affected the fresh herbage yield, with the highest yield obtained from 30 cm row spacing and the lowest yield from a wide row spacing of 50 or 60 cm. This showed that increased row spacing decreased the fresh herbage yield. Again the results differ from those of Tansı (1991), who obtained a fresh herbage yield of 2001 kg m^{-2} for *T. spicata,* primarily due to the effects of the different environments in which the experiments were conducted.

The highest dry herbage yields were also obtained from a row spacing of 30 cm and the lowest from wide spacings of 50 and 60 cm, which had the lowest populations. This is due to the increase in vegetative coverage of plants in high populations, resulting in the better use of light for photosynthesis. The dry herbage yield values varied significantly between the years, depending on the row spacing and in-rows. Differences between years might be due to the effect of precipitation and narrow row spacing and in-rows, which resulted in more yield compared to wider rows.

Herbage yield was affected by agricultural practices such as irrigation, fertilizer (Kızıl and Tonçer, 2003), harvest date (Tansı, 1991) and plant density (Badi et al., 2004). It is assumed that cutting and pruning promote branching in perennial plants. Herbage and oil production are affected by the number of plants per unit area. In the present study, fresh herbage, dry herbage, dry leaf and essential oil yield were higher in the second year than in the first year, because of the effects of cutting and harvesting in the first year.

Row spacing and in-rows significantly affected the dry leaf yield. Among the row spacings, the highest dry leaf yield was obtained from 30 cm row spacing and for in-rows the highest yield was obtained from 20 cm. This clearly shows that narrow row spacing and in-rows produced higher yield than wider rows and in-rows.

Table 2
Plant height (cm), fresh and dry herbage yield (kg m^{-2}) obtained from the trial in 2002 and 2003

Plant density (cm)		Plant height			Fresh herbage yield			Dry herbage yield		
		2002	2003	Mean	2002	2003	Mean	2002	2003	Mean
	20	33.33	34.66	34.00	1.573	1.783	1.678	0.432	0.476	0.454
30	30	33.93	37.46	35.70	1.181	1.662	1.422	0.328	0.429	0.379
	40	32.00	37.76	34.88	1.108	1.469	1.289	0.296	0.385	0.340
Mean		33.08	36.56	34.86	1.287	1.638	1.463a	0.352	0.429	0.391a
	20	32.93	39.20	34.06	1.256	1.678	1.467	0.291	0.457	0.374
40	30	33.60	37.33	35.46	1.047	1.415	1.231	0.244	0.358	0.301
	40	34.66	37.73	36.20	0.971	1.236	1.103	0.247	0.319	0.283
Mean		33.73	36.63	35.91	1.091	1.443	1.267 b	0.261	0.378	0.319 b
	20	33.86	39.00	36.43	1.019	1.587	1.303	0.272	0.389	0.331
50	30	33.33	38.40	35.86	0.954	1.188	1.071	0.245	0.305	0.275
	40	34.33	38.46	36.40	0.975	0.902	0.939	0.255	0.225	0.240
Mean		33.84	38.62	36.23	0.983	1.226	1.104 c	0.257	0.306	0.282bc
	20	35.13	37.26	36.20	0.922	1.196	1.059	0.246	0.317	0.282
60	30	36.73	37.46	37.10	1.056	1.023	1.039	0.251	0.259	0.255
	40	37.20	38.00	37.60	0.878	0.884	0.881	0.189	0.239	0.214
Mean		36.36	37.57	36.97	0.952	1.035	0.993 c	0.229	0.272	0.250 c
Mean (years)		34.25 b	37.73 a		1.078 b	1.335 a		0.275 b	0.347 a	
In-Rows	20	33.81	37.53	35.67	1.193	1.561	1.377 a	0.310	0.409	0.359 a
	30	34.40	37.66	36.03	1.059	1.322	1.192 b	0.267	0.338	0.303 b
	40	34.55	37.99	36.27	0.983	1.124	1.053 c	0.247	0.292	0.269 b
$LSD_{0.05\%}$		Years: 1.289			Years: 0.102 Row spacings: 0.144 In-rows: 0.130			Years: 0.031 Row spacings: 0.043 In-rows: 0.356		

*Means followed by the same letter are not significantly different according to LSD at the 0.05 level

The essential oil content of *T. spicata* was not affected by the plant density and ranged from 1.46–2.00%. Various ranges of essential oil content were reported by Tansı (1991) (2.48–2.64%), Tümen et al. (1994) (0.5–3.4%), Başer (2002) (1.0–3.4%) and Hanci et al. (2003) (1.57%).

The oil content of wild-grown plants is unstable, primarily due to the botanical source, age, state of plant material (fresh or dried) and the isolation technique. The essential oil contents of plants also show variations due to localities, harvesting times, temperature, duration of sunshine, air movement, rainfall and flower development stages (Özgüven and Tansı, 1998).

The essential oil yield was significantly affected by row spacing and exhibited a years × in-rows interaction. The results of the present study show that the highest oil yield was obtained from 30 cm spacing and the lowest yield from 60 cm row spacing. The interaction between years and in-rows was significant, the highest yield being obtained from 20 cm in-row spacing during the second year, and the lowest yield from 30 and 40 cm in-rows during the first year. Plant density had no significant effect on the essential oil content, but due to the increment in dry matter yield per unit area, the oil yield increased significantly at higher plant densities.

Table 3
Dry leaf yield (kg m^{-2}), essential oil content (%) and essential oil yield (L ha^{-1}) obtained from the trial in 2002 and 2003

Plant density (cm)		Dry leaf yield			Essential oil content			Essential oil yield		
		2002	2003	Mean	2002	2003	Mean	2002	2003	Mean
	20	0.299	0.347	0.323	1.66	1.92	1.79	52.4	66.3	59.3
30	30	0.228	0.311	0.269	1.58	1.92	1.75	37.1	59.3	48.2
	40	0.206	0.284	0.245	2.00	1.80	1.87	41.0	49.4	45.2
Mean		0.243	0.314	0.279 a	1.74	1.88	1.81	43.5	58.3	50.9 a
	20	0.204	0.323	0.263	1.46	1.92	1.69	29.3	61.4	45.4
40	30	0.169	0.273	0.221	1.71	1.80	1.73	29.1	47.7	38.4
	40	0.188	0.247	0.218	1.91	1.83	1.87	35.8	45.2	40.5
Mean		0.187	0.281	0.234 b	1.69	1.85	1.76	31.4	51.4	41.4 ab
	20	0.189	0.273	0.231	1.66	1.80	1.70	31.5	46.6	39.0
50	30	0.173	0.222	0.198	1.91	1.83	1.87	33.0	40.9	36.9
	40	0.176	0.176	0.176	1.83	1.92	1.87	31.3	33.5	32.4
Mean		0.179	0.224	0.202 c	1.80	1.85	1.83	31.9	40.3	36.1 b
	20	0.169	0.227	0.198	1.83	1.92	1.87	30.9	43.8	37.3
60	30	0.175	0.193	0.184	1.83	1.83	1.83	31.9	35.6	33.7
	40	0.133	0.178	0.155	1.91	1.92	1.91	26.1	33.5	29.8
Mean		0.159	0.199	0.179 c	1.85	1.89	1.87	29.6	37.6	33.6 b
Mean (years)		0.192 b	0.255 a		1.78	1.85		34.1 b	46.9 a	
In-Rows	20	0.215	0.292	0.254 a	1.65	1.88	1.76	36.0 cd	54.5 a	45.3
	30	0.187	0.249	0.218 b	1.76	1.83	1.79	32.8 d	45.9 b	39.3
	40	0.176	0.221	0.198 b	1.91	1.85	1.88	33.5 d	40.4 bc	36.9
$LSD_{0.05\%}$		Years: 0.022 Row spacings: 0.032 In-rows: 0.022			NS			Years: 7.086 Row spacings: 10.02 In-rows: 6.328		

*Means followed by the same letter are not significantly different according to LSD at the 0.05 level; NS: non-significant

Conclusions

In spite of the importance of *T. spicata* var. *spicata*, there have been very few studies on the agronomic characteristics which might be useful in improving its yield. It has the potential for high productivity, as regards both herb and oil content. A careful appraisal of its characteristics may help in accurate evaluation of the benefits.

Experiments involving replicates over years allowed a better estimation of experimental error, allowing the verification of the quality of each trial over years. Furthermore, a comparison between the climatic data in the test years and the long-term data helped to verify the results. It was concluded that narrow row spacings had a significant effect on the dry herbage yield. Moreover, 30 cm row spacing and 20 cm in-row spacing gave the best fresh and dry herbage yield, dry leaf yield and essential oil yield. High yield stability across a wide range of environments and over seasons is important, because it provides the opportunity

to maximise the yield potential. Further studies are needed to breed genetically better strains for the commercial cultivation of *T. spicata* var. *spicata* based on essential oil content and components.

Acknowledgements

This work was supported by a grant (TOG-TAG 2654) from the Scientific and Technical Research Council of Turkey (TUBITAK).

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Acta Agronomica Hungarica, 53(4), pp. 423–432 (2005)

EVALUATION OF REGENERATION POTENTIAL IN PEARL MILLET

R. KUMAR, P. SAGAR and R. K. BEHL

DEPARTMENT OF PLANT BREEDING, CCS HARYANA AGRICULTURAL UNIVERSITY, HISAR, INDIA

Received: 29 April, 2005; accepted: 28 October, 2005

The regeneration potential of pearl millet, a crop which is of vital importance for farmers in semi-arid tropical regions, was evaluated in a 12 × 12 line × tester cross involving six male sterile (A) lines from five systems of cytoplasmic-genic male sterility, namely two male sterile lines from system A_1 ($MS81A_1$, $MS8A_1$), and one each from A_2 ($Pb313A_2$), A_3 ($Pb402A_3$), A_4 ($MS81A_4$) and A_5 ($MS81A_5$), the six corresponding maintainer (B) lines $81B_1$, $8B_1$, $Pb313B_2$, $Pb402B_3$, $81B_4$ and $81B_5$, and twelve restorer (R) lines, namely H90/4-5, H77/833-2, G73-107, CSSC46-2, 77/245, 78/711, 77/273, ICR 161, ISK48, 77/28-2, 77/180 and Raj 42. The 24 parents and 144 crosses were grown separately in contiguous blocks in a randomized block design with two replications in three treatments each in 2000 and 2001 at the Research Farm, Bajra Section, Department of Plant Breeding, CCS HAU, Hisar. The plot size was 2.5 m × 0.45 m with 10 cm intra-row spacing.

The genotypes (A lines, B lines, R lines, A × R crosses, B × R crosses), sowing dates, years, year × sowing date and genotype × treatment interactions (direct and reciprocal) exhibited significant differences. The comparison of mean performance of the early-sown non-ratooned crop vs. ratooned (cut, regenerated) vs. late-sown unratooned crop treatments revealed that both the grain and dry fodder yields and the major yield-contributing characters exhibited little reduction in the ratoon crop, while the total tillers at maturity and the effective tillers increased in the ratoon crop in comparison to the direct-sown crop. Additional green fodder yields of 189.63 g and 144.02 g per plant, harvested 40 days after sowing (DAS) in the ratoon crops in 2000 and 2001, respectively, and similar grain and dry fodder yields emphasized the utility of cutting and regeneration in pearl millet. The ratooning ability of genotypes, assessed from the ratio of the performance of various characters in ratoon and early and late sown unratooned crops for yield and major yield components was high for a number of lines, testers and hybrids. Most of the hybrids involved one or two parents with good regeneration potential. Sufficient genetic variability was observed for regeneration and ratooning ability, which was found to be under genetic control, suggesting that regeneration potential could be incorporated in genotypes with high yield. A base population developed from promising parents and crosses can be improved by cyclic breeding.

Key words: pearl millet, ratooning ability, additional green fodder, dry fodder, grain yield

Introduction

Pearl millet [*Pennisetum glaucum* (L.) R. Br. emend Stuntz], an important food, feed and dual-purpose crop, is the lifeline of farms dominated by animal husbandry in the semi-arid tropics, especially on dry areas in India and Africa. It is generally cultivated under conditions of severe heat and drought in arid and semi-arid tropics. In most cases a single crop is produced under rainfed

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conditions. Its productivity is greatly dependent upon the quantity and distribution of rainfall during the crop season. It ranks fourth among the cereals, after rice, wheat and maize, and is grown on about 9.30 m ha with an annual production of 6.90 m tons in India, accounting for 31% of the area and 22% of the production of coarse cereals (1997–2001 average) (Anon., 2002).

The land holding in India being small, the farmers cannot afford to keep a part of their land exclusively for fodder purposes. Fodder, especially green, is scarce during summer. Farmers sometimes sow the crop early after the first rain (pre-monsoon showers – May, June) to cover the risk of there being no rain at the recommended sowing time. Such early-sown crops are often subject to intermittent drought if the rains fail to come. There also seems to be some possibility of delayed earing. Therefore, a method is required which would mitigate these problems to some extent making green fodder available for the cattle at the time of scarcity, and grain for human consumption. When sowing the crop 2–3 weeks earlier than the recommended time (last week of June to mid-July), harvesting the green fodder at the boot leaf stage at an appropriate stubble height (12 cm) and allowing it to regenerate to produce a grain yield, a practice known as ratooning, it is important that the anthesis period should not coincide with the rains, which however help regeneration and the production of a reasonable grain yield.

Plants in the *Gramineae* family, including pearl millet, are endowed with regeneration potential (Swanson, 1935; Hubbard and Harper, 1949; Day et al., 1968; Kawahara et al., 1998). The regeneration ability of pearl millet was studied by many authors (Burger and Hittle, 1967; Tomer and Saini, 1979; Dua and Devdas, 1985; Mangath, 1987; Suma Bai and Suresh, 1999; Desale et al., 2000).

It is not yet clear whether genotypic differences in the regeneration potential of pearl millet are heritable and whether breeding for regeneration potential is possible. An effort has been made in this study to enquire about the potential of pearl millet as a dual-purpose crop and to determine the genotypic regeneration ability, while assessing whether an early-sown crop would yield additional green fodder without a loss of grain and final dry fodder yield.

Materials and methods

The material for the present study consisted of six male sterile (A) lines from five systems of cytoplasmic-genic male sterility, namely two male sterile lines from system A_1 ($MS81A_1$, $HMS8A_1$) and one each from A_2 ($Pb313A_2$), A_3 ($Pb402A_3$), A_4 ($MS81A_4$) and A_5 ($MS81A_5$), their corresponding maintainer (B) lines ($81B_1$, $HMS8B_1$, $Pb313B_2$, $Pb402B_3$, $81B_4$ and $81B_5$) and twelve restorer (R) lines (H90/4-5, H77/833-2, G73-107, 77/245, 77/273, CSSC 46-2, ISK 48, ICR 161, 77/180, 78/711, 77/28-2 and Raj 42).

The six male sterile lines and their corresponding six maintainer lines were crossed with the twelve restorer lines in a line × tester design at ICRISAT, Hyderabad, during the off-season (January–April, 2000). The 144 hybrids thus produced and their parents were grown separately in contiguous blocks in a randomized block design with two replications in three treatments, at the Research Farm, Bajra Section, Department of Plant Breeding, CCS HAU, Hisar. The early-sown non-ratooned crop and the ratoon crop were sown on 5th June in 2000 and 25th June in 2001. The ratoon crop was cut at a height of approx. 12 cm on 14th July 2000 and 5th August 2001, and left to

regenerate. The late-sown non-ratooned treatment was sown on 14th July 2000 and 5th August 2001. The plot size was 2.5 m × 0.45 m with 10 cm intra-row spacing. All the recommended agronomic practices were followed to raise a good crop.

Observations were made on five randomly chosen, competitive plants in each plot in each replication. Three characters (plant height, total tillers/plant and green fodder yield/plant) were recorded in the vegetative stage, 40 days after sowing (DAS). In the ratooned treatment the green fodder was then cut at a stubble height of 12 cm and the stubble was allowed to regenerate. Plants in the non-ratooned treatments were not cut. Days to flowering was recorded when 50% of the plants had reached the protogyny (stigma emergence) stage. Fourteen other characters, namely number of leaves per main tiller, leaf length (cm), leaf breadth (cm), total tillers per plant (no.), effective tillers per plant (no.), plant height (cm), ear length (cm), ear diameter (mm), stem diameter (mm), 500-grain weight (g), grain yield per plant (g), dry fodder yield per plant (g), harvest index (%) = grain yield/biological yield × 100 (Donald, 1962), and growth rate (g/plant/day) = dry fodder yield (g/plant)/(days to 50 % flowering + 10) (Bramel-Cox et al., 1984), were recorded at or after maturity. Data averaged over five plants in each replication in all treatments were used for the statistical analysis. Analysis of variance for a randomized block design was carried out for each character in each of the treatments according to Federer (1977) and combined analysis of variance according to Young and Virmani (1990).

Results

Differences between the genotypes (A lines, B lines, R lines, A × R crosses, B × R crosses, direct and reciprocal), sowing dates, years, treatments, year × sowing date and genotype × treatment interactions were significant both for the individual treatments and combined analysis of variance, except for the non-significant year variation for ear length (data not presented).

As the performance trends of the various types of genotypes in the six treatments were more or less similar, for brevity only population means are explained (Table 1). The significance of differences between the treatment means was examined using the Z test. In general, the performance was better for most of the characters in the year 2000 and in early-sown, non-ratooned crops in both the years. The highest grain and dry fodder yields were obtained in the early-sown non-ratooned crop in both years and the lowest in the late-sown non-ratooned crop in 2000 and in the ratoon crop in 2001, but the differences were non-significant.

In both years flowering was significantly earlier in the late-sown non-ratooned crop than in the early-sown non-ratooned crop, but it was significantly delayed in the ratoon crop. This was not surprising, since the plants were cut at 40 DAS and regenerated plants naturally flower later. A comparison of plant height at 40 days showed that it was similar in all the early-sown treatments, but a little higher in the late-sown crop. The plant height at maturity was significantly reduced in the ratoon crop in both years. The total number of tillers at 40 days and at maturity exhibited a similar trend in both years, but there was a numerical increase in the total tillers at maturity and in the effective tillers in the ratoon crop. The number of leaves on the main stem decreased significantly in the ratoon crop in comparison to the early-sown non-ratooned crop in both years. Significantly greater ear length was recorded in the early-sown non-ratooned crop than in the ratoon crop in both years, while the ratoon crop and the late-sown non-ratooned crop were statistically at par. The greatest leaf length and

leaf breadth were recorded in the early-sown non-ratooned crop in both years and the lowest in the ratoon crop, but the trend for the different treatments was the same in both years. In 2000 ear diameter and stem diameter were greatest in the early-sown non-ratooned crop, and a significant reduction was recorded for both characters in the ratoon crop. A significant reduction was also found for the stem diameter in the ratoon crop and the late-sown crop in 2001. Heavier seeds were obtained in the late-sown non-ratooned crop than in the ratoon crop in both years. The harvest index showed similar values in all the treatments, but the highest percentage was recorded in the early-sown non-ratooned crop in 2000 and in the ratoon crop in 2001. The lowest growth rate was recorded in the ratoon crop in both years.

The population means in the ratoon crop in the two years showed a mixed trend, but were mostly lower, with the exception of a few characters such as total tillers at maturity and effective tillers. A comparison of the ratoon crop and the late-sown unratooned crop in the two years showed that the mean number of leaves per main tiller and the mean stem diameter were significantly lower in 2000 and the mean leaf length, mean leaf breadth and mean growth rate in 2001, but for all the other characters the performance was at par. The green fodder yield (cut at 40 days) was 189.63 g per plant in 2000 and 144.02 g per plant in 2001. The difference was non-significant, indicating that the early-sown crop had the advantage of giving additional green fodder. After cutting at the boot leaf stage and regeneration it gave a similar grain and dry fodder yield, emphasizing the utility of cutting and regeneration in pearl millet.

Table 1

Treatment means of genotypes for some quantitative characters in the experimental treatments

Characters	2000				2001				Overall mean
	E1	E2	E3	Mean	E1	E2	E3	Mean	
Grain yield (g/plant)	30.94	26.11	23.19	26.75	29.17	24.53	24.65	26.12	26.40
Dry fodder yield at maturity^{+}	76.46	72.89	60.85	70.07	72.89	60.23	64.56	65.89	67.98
Green fodder yield at 40 days^{+}	–	189.63	–	–	–	144.02	–	–	166.82
Days to 50% flowering	51.33	74.82	46.99	57.71	58.60	75.21	50.06	61.29	59.50
Plant height at 40 days (cm)	89.49	88.48	105.16	94.37	86.01	87.37	96.41	89.93	92.15
Plant height at maturity (cm)	205.19	177.45	190.59	191.08	199.67	163.72	176.47	179.95	185.51
Total tillers at 40 days^{++}	3.71	3.83	3.07	3.54	3.74	3.57	3.82	3.71	3.62
Total tillers at maturity^{++}	3.73	4.24	3.11	3.70	3.81	4.22	4.23	4.09	3.89
Effective tillers (No./plant)	2.12	2.34	2.04	2.17	2.06	2.71	2.25	2.34	2.25
No. of leaves/main tiller	8.94	6.63	8.56	8.05	8.96	6.48	7.39	7.61	7.83
Leaf length (cm)	60.94	50.72	55.03	55.57	60.88	49.17	55.36	55.14	55.38
Leaf breadth (cm)	3.87	3.09	3.39	3.45	3.84	2.91	3.43	3.39	3.43
Ear length (cm)	26.45	21.92	24.06	24.14	25.87	22.71	23.86	24.15	24.15
Ear diameter (mm)	23.09	19.78	21.49	21.45	22.47	20.67	21.61	21.58	21.52
Stem diameter (mm)	8.09	7.23	7.85	7.72	8.13	7.18	7.24	7.52	7.62
500-grain weight (g)	4.27	4.06	4.41	4.24	4.23	3.96	4.25	4.15	4.20
Harvest index (%)	28.61	26.03	27.30	27.31	28.17	28.51	27.19	27.96	27.64
Growth rate (g/plant/day)	1.25	0.86	1.07	1.06	1.06	0.71	1.08	1.08	1.01

E1= early-sown non-ratooned crop, E2= ratoon crop, E3= late-sown non-ratooned crop, $^{+}$= g/plant, $^{++}$= No./plant

The ratooning ability of the genotypes was assessed from the ratio of the performance of various characters in treatments involving ratooning to that in early-sown or late-sown treatments without ratooning in both years. The results are presented in Tables 2a and b. Genotypes with a ratio of ≥ 1.0 for grain yield, dry fodder yield, plant height and effective tillers were considered to have efficient regeneration. Lines 3 ($313A_2$), 1 ($81A_1$), 5 ($81A_4$), 6 ($81A_5$) and 7 ($81B_1$) and testers 17 (77/245), 13 (H90/4-5), 19 (77/273), 21 (ISK 48) and 24 (Raj-42) exhibited high regeneration potential. The hybrids 1 × 13, 1 × 18, 1 × 19, 2 × 18, 2 × 22, 4 × 14, 6 × 13, 7 × 18, 7 × 24, 9 × 13, 9 × 22, 10 × 14, 10 × 19, 10 × 20, 11 × 14, 11 × 16, 12 × 15, 12 × 16 and 12 × 19 also had superior regeneration potential. Most of these hybrids involved one or two parents with good regeneration potential, indicating that the trait was under genetic control.

Discussion

All the genotypes exhibited regeneration in both years, but there was wide variability among the genotypes. A comparison of the mean performance for the various characters studied in the two years revealed that both grain and dry fodder yield and major yield components such as plant height, number of leaves, leaf length, leaf breadth, ear length, stem diameter, growth rate and 500-grain weight, exhibited little reduction in the ratoon crop in comparison to the early-sown and late-sown non-ratooned crops, but the total and effective tiller numbers and the days to flowering increased in the ratoon crop. A reduction in the yield and yield components and an increase in the tiller number after ratooning were also found by Bainiwal (1982) in barley and Mangath (1987) in pearl millet. The increase or maintenance of the yield after ratooning could be due to the better regeneration rate, expressed as the ability of the sward to regenerate new photosynthetic tissue, with maximum merismatic activity and efficient translocation of the photosynthates to the growing apex (Kamidi and Wanjala, 1988).

Though no scientific reasons have yet been put forward to explain the regeneration of pearl millet, in other crops a number of reasons have been postulated such as the preponderance of amylase activity over acid and neutral invertases to provide hexoses to allow the stubble buds to sprout (Kumar and Bhatnagar, 1992), or total non-structural carbohydrate concentrations in belowground parts, associated with regrowth and perenniality in rice (Turner and Jund, 1993) and forage crops (Sheaffer et al., 1988). Sprague and Sullivan (1950) found large amounts of carbohydrates stored in the lower leaf sheath and stubble of orchard grass and rye grass, part of which is removed with the cut, while the rest is utilized for vegetative growth. Accumulated photosynthates were indicated as the energy resource for the initiation of regeneration growth after ratooning by Kawahara et al. (1998) in rice. Ichii and Sumi (1983) found a strong correlation between the total available carbohydrate content in the stem and the stem base weight, and ratooning traits appeared to depend largely on these two characters.

Table 2a
Ratios of grain yield/plant and dry fodder yield/plant in ratoon crops compared with early-sown and late-sown non-ratooned crops

No.	Genotype	Grain yield/plant (g)				Dry fodder yield/plant (g)			
		2000		2001		2000		2001	
		a	b	a	b	a	b	a	b
	Lines								
1	81A1	0.98	1.68	1.34	1.12	1.11	1.90	1.14	0.91
2	8A1	0.75	1.37	0.78	0.78	0.84	1.64	0.96	0.69
3	313A2	1.05	2.31	1.67	1.90	1.22	1.74	1.32	1.35
4	402A3	0.60	0.74	1.20	1.66	0.83	1.04	1.07	1.22
5	81A4	1.25	1.98	1.93	1.55	1.01	1.44	1.70	1.20
6	81A5	0.80	1.50	1.06	1.55	0.79	1.26	1.01	1.04
7	81B1	1.06	2.20	0.97	1.17	0.99	1.71	1.04	0.97
8	8B1	0.73	1.35	0.90	0.89	0.77	1.36	1.03	0.82
9	313B2	0.53	1.37	0.84	0.97	0.86	1.34	1.06	1.03
10	402B3	0.49	0.80	0.93	0.86	0.79	1.41	0.77	0.71
11	81B4	0.97	1.56	1.39	1.24	1.03	1.48	1.78	1.14
12	81B5	0.99	1.79	1.52	1.29	1.05	1.49	1.52	1.44
	Testers								
13	H90/4-5	0.96	1.36	1.78	1.62	1.58	1.30	1.63	1.60
14	H77/833-2	0.37	0.66	0.63	1.18	0.56	0.92	0.67	0.97
15	G73-107	0.95	1.11	0.94	0.74	1.24	0.89	1.26	0.78
16	CSSC46-2	0.77	1.48	0.73	0.84	1.40	1.86	0.85	0.96
17	77/245	1.02	1.53	1.17	2.40	1.32	1.57	1.15	1.16
18	78/711	0.58	0.61	1.39	1.07	0.54	0.71	1.39	1.94
19	77/273	0.95	1.03	1.71	1.20	1.24	1.42	1.73	1.13
20	ICR-161	0.60	1.17	0.74	0.72	1.30	1.63	0.95	0.69
21	ISK-48	0.73	1.32	1.19	1.27	0.98	1.34	1.11	1.28
22	77/28-2	0.60	0.92	1.25	0.95	0.81	1.12	0.94	0.98
23	77/180	0.74	1.26	0.71	0.74	1.04	1.47	0.92	1.09
24	Raj-42	0.75	1.12	1.82	1.38	1.57	1.99	1.86	1.86
	Hybrids								
1	1×13	1.14	1.46	0.89	1.10	1.04	1.42	0.91	1.16
6	1×18	1.44	1.13	1.54	1.00	1.44	1.42	1.24	0.91
7	1×19	1.03	1.18	1.07	0.91	1.12	1.11	1.26	1.13
18	2×18	1.36	1.92	0.95	1.48	1.20	1.20	1.04	1.31
22	2×22	1.05	1.27	0.98	1.20	1.10	0.94	1.28	1.35
38	4×14	1.54	1.26	2.14	1.98	1.76	1.20	1.35	0.83
61	6×13	1.17	1.54	1.00	1.13	1.23	1.60	1.05	1.23
78	7×18	1.18	1.44	1.10	1.36	1.03	1.39	0.97	0.84
84	7×24	1.33	1.72	1.50	1.15	0.86	1.45	1.28	1.10
97	9×13	1.24	1.20	1.02	0.97	1.15	1.29	0.96	1.04
106	9×22	1.23	1.10	2.17	1.24	0.99	1.04	1.11	1.20
110	10×14	1.30	1.34	0.98	1.15	1.40	1.28	1.07	0.71
115	10×19	1.04	1.19	2.24	1.47	1.37	1.35	1.08	0.91
116	10×20	1.14	1.09	1.06	1.12	1.17	1.25	0.82	0.91
122	11×14	1.20	1.31	1.02	1.19	1.20	1.01	1.02	1.11
124	11×16	1.06	1.28	1.60	1.51	1.01	1.58	1.00	1.02
135	12×15	1.15	1.29	1.23	1.30	1.25	1.19	1.12	1.16
136	12×16	1.02	1.25	1.11	2.06	1.21	1.43	1.12	1.66
139	12×19	1.00	0.81	1.09	1.54	0.91	1.00	0.81	1.37

a=E2:E1; b=E2:E3; E1= early-sown non-ratooned crop; E2= ratoon crop; E3=late sown non-ratooned crop

Table 2b
Ratios of plant height at maturity and effective tillers in ratoon crops compared with early-sown and late-sown non-ratooned crops

No.	Genotype	Plant height at maturity (cm)				Effective tillers (No./plant)			
		2000		2001		2000		2001	
		a	b	a	b	a	b	a	b
	Lines								
1	81A1	0.81	0.91	1.06	1.00	0.89	1.26	2.11	1.60
2	8A1	0.81	0.95	1.02	1.03	1.08	1.75	1.50	1.14
3	313A2	1.01	0.94	1.02	1.37	1.05	1.47	1.65	2.32
4	402A3	0.83	0.85	0.93	1.02	1.21	1.10	1.76	1.50
5	81A4	0.87	0.92	0.88	1.00	1.26	2.23	1.08	1.17
6	81A5	0.91	0.90	0.99	1.03	1.30	2.17	2.41	2.05
7	81B1	0.80	0.90	1.01	1.01	1.08	1.44	1.07	1.35
8	8B1	1.02	1.05	1.01	0.93	1.08	1.47	1.00	1.17
9	313B2	0.78	0.77	0.92	1.12	1.25	1.25	1.08	1.30
10	402B3	0.88	0.85	1.03	1.08	1.29	1.17	0.92	1.00
11	81B4	1.04	0.86	0.82	0.93	0.92	0.77	0.81	1.00
12	81B5	0.95	0.96	1.03	1.04	1.08	1.13	1.48	1.82
	Testers								
13	H90/4-5	0.91	0.86	0.85	1.10	0.86	1.14	1.39	1.28
14	H77/833-2	0.78	0.84	0.83	0.90	0.80	0.62	0.58	0.62
15	G73-107	0.81	0.81	0.98	0.94	0.88	0.78	1.25	0.94
16	CSSC46-2	0.88	1.01	0.81	0.82	1.44	1.53	1.18	1.18
17	77/245	0.94	1.00	0.96	1.05	1.16	1.21	1.68	1.37
18	78/711	0.77	0.99	0.98	1.23	1.08	1.22	1.52	1.52
19	77/273	1.08	0.98	0.91	0.88	1.12	1.26	2.18	1.48
20	ICR-161	0.86	1.01	0.83	0.86	1.18	1.05	1.27	0.86
21	ISK-48	1.01	1.04	0.85	0.99	0.96	0.79	2.06	1.67
22	77/28-2	1.02	1.01	0.93	0.87	1.00	0.83	1.31	0.84
23	77/180	0.79	0.96	0.64	1.02	1.08	1.23	1.67	1.48
24	Raj-42	0.97	1.30	1.23	1.26	0.84	0.70	0.68	0.85
	Hybrids								
1	1×13	0.77	0.95	0.82	0.99	1.36	1.62	1.30	1.30
6	1×18	0.99	1.06	0.89	0.89	0.76	0.89	0.90	0.64
7	1×19	0.78	0.84	0.82	0.90	0.86	1.06	1.76	1.42
18	2×18	0.96	0.98	0.86	0.87	1.17	1.17	0.90	1.24
22	2×22	0.76	0.90	0.75	0.89	0.81	0.81	1.79	1.89
38	4×14	0.81	0.86	0.70	0.77	1.23	1.14	2.21	0.97
61	6×13	0.83	0.99	0.80	1.09	1.60	1.88	2.38	1.63
78	7×18	0.96	0.97	0.89	0.84	1.14	1.32	1.05	1.05
84	7×24	0.84	0.99	0.82	1.10	1.10	1.57	0.95	1.05
97	9×13	0.93	1.01	0.86	1.11	1.40	1.17	2.00	1.44
106	9×22	0.92	1.01	0.95	1.24	1.13	0.94	1.27	1.47
110	10×14	0.80	0.92	0.66	0.90	1.31	1.13	1.38	0.94
115	10×19	0.83	0.92	0.79	0.82	1.30	1.63	1.78	1.03
116	10×20	0.92	1.01	0.89	0.98	1.06	0.90	1.64	1.10
122	11×14	0.84	0.94	0.85	1.08	1.40	1.84	1.13	1.04
124	11×16	0.87	0.95	0.78	0.84	1.20	1.13	1.89	1.48
135	12×15	0.94	0.98	0.81	0.82	1.09	1.20	1.94	1.48
136	12×16	0.89	0.98	0.83	0.95	1.63	1.73	2.05	1.70
139	12×19	0.85	0.84	0.81	0.95	1.09	1.20	1.58	1.88

a=E2:E1; b=E2:E3; E1= early-sown non-ratooned crop; E2= ratoon crop; E3=late sown non-ratooned crop

A comparison of the ratoon crop and the late-sown non-ratooned crop on the same date showed encouraging results, as there was no reduction in the yield and yield components except for the number of leaves per main tiller and the mean stem diameter in 2000 and the mean leaf length, leaf breadth and growth rate in 2001, which showed a significant reduction. The early-sown crop cut at 40 days after sowing showed the advantage of giving similar grain and fodder yields after regeneration. This was mainly due to the maintenance of total and effective tiller numbers.

An appreciably higher grain yield in pearl millet crops cut 35 days after sowing in comparison to the non-ratooned crop was reported by Tomer and Saini (1979). Day et al. (1968) and Skorda (1977) also reported an increase in grain yield after cutting in barley. Thus, the practice of sowing the crop early, some time in June, to reduce the risk of the rain not arriving in time, and regenerating the crop for grain yield is advantageous. The ratoonability of pearl millet adds a degree of versatility that could be useful if the monsoon fails. Ratooning saves the cost of establishing a new crop, while making green fodder available at a time of scarcity.

The high regeneration potential, judged on the basis of *per se* ratoonability potential and percentage gain in biomass, revealed high environmental inconsistency among the top 10 rankers as far as gain was concerned. Moreover, regeneration potential and *per se* performance appeared to be independent of each other, as genotypes were found with low regeneration potential and higher yield (lines $Pb402A_3$, $Pb402B_3$, $HMS8A_1$ and $HMS8B_1$, testers CSSC46-2 and 77/180, and hybrids 8 × 16, 3 × 18, 5 × 17, 11 × 17) and *vice versa* (lines $Pb313A_2$, $Pb313B_2$, $MS81A_4$ and $MS81B_4$, and testers H90/4-5 and 77/273). However, lines 313 A_2, and $81A_4$, testers H90/4-5 and 77/245 and hybrids 4 × 14, 6 × 13 and 9 × 13 were ranked in the top ten in all four comparisons, while hybrids 1 × 13, 1 × 18, 10 × 19, 11 × 16 and 12 × 16 were in the top ten in three of the four comparisons. High grain yield could mainly be attributed to a high number of effective tillers per plant. Both parents of a hybrid showing high regeneration potential need not necessarily satisfy these criteria. Nevertheless, the merit of hybrid 1 × 13 ($81A_1$ × H90/4-5 = HHB-50), released for general cultivation in India in 1987, which is not only one of the best yielders in India, but also has high regeneration potential, is based on a specific combination of parents, particularly the restorer H90/4-5, which has given high gain and is also a good yielder and a good general combiner for a number of characters, including grain yield. The hybrids 9 ×13 ($313B_2$ × H90/4-5) and 12 × 16 ($81B_5$ × CSSC 46-2) involve at least one parent with good gain, which is encouraging and warrants further confirmation. The hybrid 4 × 14 ($402A_3$ × H77/833-2), though involving parents with low gain, shows that dominant and desirable allelic interactions are operative in the expression of regeneration potential. Ratoonability was reported to be genetically controlled in rice (Li and Chen, 1988; Chauhan et al., 1989; 1991; Arumugachami et al., 1995) and in maize,

teosinte and their hybrids (Shieh and Lu, 1995). Both additive and non-additive gene effects were reported in the expression of ratoonability in rice (Chauhan et al., 1991) and in pearl millet (Kumar, 2002). The present results show that (a) genetic variability for regeneration potential exists in pearl millet genotypes, (b) regeneration potential is a heritable trait, (c) some genotypes ($MS81A_5$, $Pb313A_2$, $MS81A_1$, 77/245, ISK48, ICR 161) appear to be general combiners, as their involvement in crosses leads to enhanced regeneration, (d) specific cross combinations or populations emanating from crosses between hybrids 1 × 13, 1 × 18, 10 × 19, 11 × 16, 12 × 16 and 4 × 14 and parents 3, 5, 13, 17 and 19 might result in the accumulation of favourable alleles for *per se* performance and high yield. This base population could be improved for yield and regeneration potential by cyclic breeding. Also, a three-way cross involving parents (3 or 5 × 13) × 17 is expected to yield high heterosis and good transgressive segregants. Thus, the combination of high regeneration potential in dual-purpose genotype(s) is a distinct possibility, and plant breeding options to tailor such genotypes must be exploited.

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Acta Agronomica Hungarica, 53(4), pp. 433–437 (2005)

Short communication

EFFECT OF SALICYLIC ACID ON GROWTH AND ENZYME ACTIVITIES OF WHEAT SEEDLINGS

S. HAYAT, Q. FARIDUDDIN, B. ALI and A. AHMAD

PLANT PHYSIOLOGY SECTION, DEPARTMENT OF BOTANY,
ALIGARH MUSLIM UNIVERSITY, ALIGARH, INDIA

Received: 5 November, 2004; accepted: 21 October, 2005

Grains of wheat (*Triticum aestivum* L. cv. Raj-3077) were soaked in 0, 10^{-5}, 10^{-4} or 10^{-3} M aqueous solutions of salicylic acid (SA) for 3, 6 or 9 h. The seedlings raised from grains pre-treated with 10^{-5} M SA possessed significantly higher leaf number, fresh and dry mass per plant, and nitrate reductase and carbonic anhydrase activities 30 and 40 days after sowing. However, 10^{-3} M SA reduced all the above-mentioned parameters.

Key words: carbonic anhydrase, nitrate reductase, *Triticum aestivum*

Abbreviations: SA – salicylic acid; DAS – days after sowing; CA – carbonic anhydrase; NR – nitrate reductase

Introduction

Salicylic acid (SA) is one of the numerous phenolic compounds found in plants. Phenolics in general may function as plant growth regulators (Aberg, 1981). The exogenous application of SA to plants generates diverse physiological effects, such as the inhibition of dry mass accumulation (Schettel and Balke, 1983), the promotion of stomatal closure (Larque-Saavedra, 1979) and the inhibition of ethylene synthesis (Leslie and Romani, 1986). SA is involved in the initiation of an alternative respiratory pathway (Elthon et al., 1989) through the regulation of a specific nuclear gene encoding the alternative oxidase protein in *Sauromatum guttatum* (Rhoads and McIntosh, 1991). SA plays an important role in flower induction, growth and development, ethylene biosynthesis, stomatal behaviour and respiration (Raskin, 1992). Young maize plants exhibited increased cold tolerance upon treatment with SA, aspirin or benzoic acid (Janda et al., 1999; 2000). Exogenously added SA also increased the heat tolerance of mustard (Dat et al., 2000). Moreover, SA inhibited the synthesis of total soluble protein, the synthesis of ribulose-1,5-bisphosphate carboxylase/oxygenase, Rubisco (Pancheva and Popova, 1998) and Hill activity, and minor changes were reported in the kinetics of O_2 evolution (Maslenkova and Toncheva, 1998) in association with the changes in net photosynthetic rate, chlorophyll and nitrate reductase activity (Fariduddin et al., 2003). The growth of barley seedlings improved when the grains were pre-treated with SA (Pancheva et al., 1996). The objective of the present study was to assess the involvement of SA in the nitrogen and carbon assimilation pathway by analysing the status of NR and CA activities in young seedlings of wheat.

0238–0161/$ 20.00

Materials and methods

The grains of wheat (*Triticum aestivum* L. cv. Raj 3077) were obtained from the National Seed Corporation Ltd., New Delhi, India. The seeds were stored in the dark at 25±2°C. Plastic pots, each filled with 500 g of acid-washed sand were used. The seeds were surface sterilized with 0.5% (v/v) sodium hypochlorite solution for about 15 min, followed by repeated washings with double distilled water. The seeds were then soaked in water (control), 10^{-5}, 10^{-4} or 10^{-3} M aqueous solutions of salicylic acid (SA) for 3, 6 or 9 h. Five soaked seeds were sown per pot and each treatment was replicated five times. They were supplied with 50 cm^3 of nutrient solution (Hoagland and Arnon, 1950) on alternate days. The whole experiment was conducted twice. The experiments were conducted under controlled conditions (light intensity of 12 W m^{-2}, photoperiod 14 h and temperature 25±2°C). Leaf number, fresh and dry mass per plant, and the nitrate reductase (NR) and carbonic anhydrase (CA) activities in the leaves were determined on the 30th and 40th day after sowing (DAS). The activity of NR was measured by the method of Jaworski (1971). The procedure described by Dwivedi and Randhawa (1974) was followed for CA activity. The data were statistically analysed following the method described by Gomez and Gomez (1984).

Results and discussion

The seedlings raised from grains treated with the lowest concentration (10^{-5} M) of SA possessed significantly higher leaf number, fresh and dry mass per plant (Tables 1 and 2a, b) as compared with the control, sampled at 30 and 40 DAS. The leaves of these seedlings also exhibited higher NR and CA activities (Table 3a, b). However, the values decreased as the concentration of SA was increased and fell below that of the control at the maximum concentration (10^{-3} M). The increased activity of NR at a dilute concentration (10^{-5} M) of SA could have been either the expression of an interaction between the acid and specific inhibitors, whose presence is claimed by Srivastava (1980), and/or due to the mediation of other hormones. Auxins are protected by phenols (Schneider and Whitman, 1974) and elevate the activity of NR (Ahmad, 1988). Moreover, the concentration of any active protein (enzyme) represents a fine balance between its synthesis/activation and degradation/inactivation (Jain and Srivastava, 1981). The concentration of SA might play an active role in regulation, where the lower concentration favoured an increase in NR protein and a higher quantity of SA decreased it by affecting the above processes. Similar results were also reported by Fariduddin et al. (2003) in mustard leaves.

The activity of carbonic anhydrase was also significantly higher in the leaves of seedlings raised from grains treated with SA (Table 3a, b). These results support the findings of Fariduddin et al. (2003) in mustard leaves. Among the other hormones, cytokinin (Sugiharto et al., 1992) and homobrassinolide (Hayat et al., 2000; 2001) enhanced the activity of CA in the leaves of maize and mustard, respectively. In the former it acts by delaying the degradation of the mRNA responsible for the synthesis of CA. SA might also have acted somewhere at the transcription and translational level in the wheat seedlings. CA activity in the leaves is naturally expected to increase the photosynthetic

Table 1
Effect of pre-sowing seed treatment with salicylic acid (SA) for different durations on leaf number in wheat, at 30 and 40 days after sowing (DAS)

Treatment	30 DAS				40 DAS			
	3 h	6 h	9 h	Mean	3 h	6 h	9 h	Mean
Control	1.20	1.60	1.60	1.46	1.80	1.90	1.90	1.86
10^{-5} M	1.65	1.90	1.93	1.82	2.20	2.60	2.60	2.46
10^{-4} M	1.30	1.69	1.71	1.56	2.00	2.00	2.00	2.00
10^{-3} M	1.00	1.25	1.25	1.16	1.60	1.60	1.60	1.60
Mean	1.28	1.61	1.62		1.9	2.02	2.02	
$LSD_{5\%}$	S = 0.08				S = 0.10			
	SA = 0.06				SA = 0.07			
	S × SA = NS				S × SA = NS			

S = soaking, NS = non-significant

Table 2a
Effect of pre-sowing seed treatment with salicylic acid (SA) for different durations on fresh mass (mg plant^{-1}) in wheat, at 30 and 40 days after sowing (DAS)

Treatment	30 DAS				40 DAS			
	3 h	6 h	9 h	Mean	3 h	6 h	9 h	Mean
Control	193	205	204	200	305	315	317	312
10^{-5} M	228	259	250	245	363	404	400	389
10^{-4} M	190	198	201	196	310	320	322	317
10^{-3} M	145	162	169	158	282	290	291	287
Mean	189	206	206		315	332	332	
$LSD_{5\%}$	S = 23				S = 22			
	SA = 11				SA = 15			
	S × SA = NS				S × SA = NS			

S = soaking, NS = non-significant

Table 2b
Effect of pre-sowing seed treatment with salicylic acid (SA) for different durations on dry mass (mg plant^{-1}) in wheat, at 30 and 40 days after sowing (DAS)

Treatment	30 DAS				40 DAS			
	3 h	6 h	9 h	Mean	3 h	6 h	9 h	Mean
Control	61	65	66	64	100	102	103	101
10^{-5} M	73	85	83	80	120	139	133	130
10^{-4} M	63	64	68	65	109	113	110	110
10^{-3} M	48	53	55	52	82	90	91	87
Mean	61	66	68		102	111	109	
$LSD_{5\%}$	S = 0.009				S = 0.008			
	SA = 0.007				SA = 0.007			
	S × SA = NS				S × SA = NS			

S = soaking, NS = non-significant

Table 3a

Effect of pre-sowing seed treatment with salicylic acid (SA) for different durations on nitrate reductase (NR) activity [n mol NO_2^{-1} g^{-1} h^{-1}] in wheat, at 30 and 40 days after sowing (DAS)

Treatment	30 DAS				40 DAS			
	3 h	6 h	9 h	Mean	3 h	6 h	9 h	Mean
Control	115	119	116	116.6	130	133	133	132
10^{-5} M	141	169	167	159	170	188	186	181
10^{-4} M	118	120	117	118.3	132	135	136	134.3
10^{-3} M	96	101	99	98.6	113	120	121	118
Mean	117.5	127.3	124.8		136	144	144	
$LSD_{5\%}$	S = 14.21				S = 11.25			
	SA = 9.05				SA = 8.73			
	S × SA = 25.03				S × SA = 20.0			

S = soaking

Table 3b

Effect of pre-sowing seed treatment with salicylic acid (SA) for different durations on carbonic anhydrase (CA) activity [mol (CO_2) kg^{-1}(fm) s^{-1}] in wheat, at 30 and 40 days after sowing (DAS)

Treatment	30 DAS				40 DAS			
	3 h	6 h	9 h	Mean	3 h	6 h	9 h	Mean
Control	1.61	1.65	1.63	1.63	1.80	1.91	1.88	1.86
10^{-5} M	1.87	2.05	2.01	1.97	2.00	2.18	2.19	2.12
10^{-4} M	1.65	1.73	1.74	1.7	1.83	1.95	1.97	1.91
10^{-3} M	1.40	1.44	1.49	1.44	1.61	1.73	1.67	1.67
Mean	1.63	1.71	1.71		1.81	1.94	1.92	
$LSD_{5\%}$	S = 0.07				S = 0.11			
	SA = 0.06				SA = 0.08			
	S × SA = 0.14				S × SA = 0.20			

S = soaking

efficiency (Everson, 1970) by maintaining a constant supply of CO_2 for reduction by Rubisco (Okabe et al., 1980). The availability of larger quantities of organic nitrogen and photosynthates will obviously lead to vigorous plant growth with increased dry weight (Table 2a,b). However, the higher concentration of SA made a permanent change at the level of the membrane organization of the cells that proved injurious for plant metabolism and growth (Tables 1 and 2a, b) (Fariduddin et al., 2003).

Acknowledgements

The financial assistance rendered by the Department of Science and Technology (S.H.) and the Council of Scientific and Industrial Research (Q.F.), New Delhi is gratefully acknowledged.

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Acta Agronomica Hungarica, 53(4), pp. 439–441 (2005)

Short communication

INHERITANCE OF AROMA IN RICE

P. C. KOLE

DEPARTMENT OF CROP IMPROVEMENT, HORTICULTURE AND AGRICULTURAL BOTANY, INSTITUTE OF AGRICULTURE, VISVA-BHARATI, SRINIKETAN, WEST BENGAL, INDIA

Received: 13 December, 2004; accepted: 28 October, 2005

The inheritance of aroma was studied in three advanced breeding lines derived from the γ-irradiation of the aromatic rice cultivar Gobindabhog. For sensing aroma, leaves were used in F_1 and F_2 plants, while kernels were tested in the F_3 generation. The results indicate that aroma in the breeding lines is controlled by a single recessive gene. The mutants retained the aroma gene, possibly unaltered, from the mother cultivar Gobindabhog.

Key words: indica rice, aroma, inheritance

Introduction

Rice quality is a combination of several physico-chemical characters. Scent in rice is an important quality character. The characteristic rice aroma is due to the presence of the volatile oil 2-acetyl-1-pyrroline, which all rice varieties have, but when it exceeds a threshold of 0.1 ppm (by weight), the variety is recognized as scented. The present investigation was conducted to study the inheritance of aroma in induced mutants, which could be potentially used in the cross-breeding of aromatic rice.

Materials and methods

Dry husked seeds of Gobindabhog were irradiated with 20 and 30 kR of γ-rays (source Co^{60}) in 1986 and following the pedigree method, a number of distinct true-breeding mutants, retaining the characteristic aroma of the mother variety, were isolated. The induced mutants 42(12)12 and 42(1)1 have sturdy stems with higher grain weight, while 21(6)3 has high panicle density. Mutants 42(12)12 and 21(6)3 are semi-dwarf, non-allelic to sd1 of DGWG, while 42(1)1 is a semi-tall type. The non-aromatic variety IET 5656 was crossed with 42(12)12 and 21(6)3 and another non-aromatic variety IR-50 was crossed with 42(1)1. In addition to these three scented vs. non-scented crosses, the mutants were also crossed with Gobindabhog. The parents, F_1s and F_2s were raised at a spacing of 30×25 cm^2 during the warm wet season at the Agriculture Farm, Institute of Agriculture, Visva-Bharati (23°39′N, 87°42′E, 58.9 masl). For the detection of aroma, 2 g of leaves collected from the upper half of the plants at flowering were cut into small pieces (2–3 mm in length) and placed on covered Petri dishes containing 10–15 ml of 0.1 N KOH solution. These were smelt after 10–15 minutes and rated for aroma (Singh et al., 1986). The aroma in F_3 kernels from individual F_2 plants was tested by a panel of three people who each chewed 20 seeds (Dhulappanavar, 1976).

Results and discussion

The F_1 plants of all the three crosses between mutants and non-scented varieties (Table 1) were non-aromatic, indicating that the presence of aroma is controlled by recessive gene(s). The observed frequencies for aromatic:non-aromatic plants in the F_2 generation were 46:154 in 42(1)1 × IR-50, 61:139 in 42(12)12 × IET 5656 and 59:141 in 21(6)3 × IET-5656, which fitted the 1:3 ratio with a probability value of 0.5–0.7, 0.05–0.10 and 0.1–0.20, respectively. This indicated that aroma in the above mutants is due to a single recessive gene. Since aroma is a characteristic of the rice endosperm, F_3 kernels produced on F_2 plants were tested for confirmation of the results. A total of 60 F_2 plants per cross were randomly selected, in which 15 aromatic plants were deliberately included with the expectation that these would be true-breeding for aroma. The expectation was quite correct in all the crosses. The remaining 45 plants segregated in a ratio of 1 true-breeding non-aromatic:2 segregating for aroma (1 aromatic:3 non-aromatic) in all the crosses, confirming the results of monogenic inheritance for aroma (Table 2). The results are in complete agreement with Berner and Hoft (1986), Nadaf et al. (1993) and Katare and Jambhale (1995). However, other genetical ratios for the presence vs. absence of scent have also been reported, namely 3:1 (Kadam and Patankar, 1938), 9:7 (Tomar and Nanda, 1983), 3:13 (Tsuzuki and Shimokawa, 1990), 1:15 (Geetha, 1994), 27:37 (Tomar and Nanda, 1983), 81:175 (Kadam and Patankar, 1938; Dhulappanavar, 1976) and 27:229 (Nadaf et al., 1993).

Table 1
Segregation pattern for aromatic and non-aromatic plants in the F_2 generation of different crosses

Cross	Total plants screened	Number of		χ^2 (3:1)	p
		aromatic plants	non-aromatic plants		
42(1)1 × IR 50	200	46	154	0.327	0.50–0.70
42(12)12 × IET 5656	200	61	139	2.940	0.05–0.10
21(6)3 × IET 5656	200	59	141	1.927	0.10–0.20

Table 2
Segregation pattern for aroma in F_3 kernels produced on non-aromatic F_2 plants of different crosses

Cross	Total plants screened	Number of families		χ^2 (1:2)	p
		Homozygous non-aromatic	Segregating		
42(1)1 × IR 50	45	11	34	1.225	0.20–0.30
42(12)12 × IET 5656	45	12	33	0.625	0.30–0.50
21(6)3 × IET 5656	45	14	31	0.025	0.8 –0.90

A study of F_1 and 100 F_2 plants in each of the crosses of mutants with their mother variety Gobindabhog showed the presence of aroma in all the plants. This indicates that the mutants retained the original aroma gene(s), possibly unaltered, of the mother variety.

The intensity of aroma varied in aromatic plants of the segregating population, especially in crosses with non-aromatic varieties. Variability in aroma quality is also possible, as scentedness depends on the quantity of 2-acetyl-1-pyrroline accumulated in the plant (Buttery et al., 1983). The character aroma has also been reported to be highly influenced by environmental variations and is stated to be low in heritability (Juliano, 1972).

Aromatic rices are prized not only because of their aroma, but also because of the extreme grain elongation on cooking of soaked milled rice and the soft texture of cooked rice. The majority of aromatic rice varieties grown in different parts of India and Pakistan are tall indica, which are susceptible to lodging and poor yielders. Therefore, high-yielding aromatic rice varieties could be developed either through the crossing of aromatic varieties followed by backcrosses with aromatic varieties to obtain other desired cooking quality characteristics or through the crossing of such useful height mutants/aromatic varieties with other aromatic varieties followed by handling the segregating population through the pedigree method. The latter method would be obviously comparatively easier. However, our experiences in crosses involving plants that are both aromatic but from diverse sources reveals the appearance of non-aromatic plants in segregating generations. However, this will not seriously hamper the progress of selection, since the frequency of non-aromatic plants in such crosses is very low.

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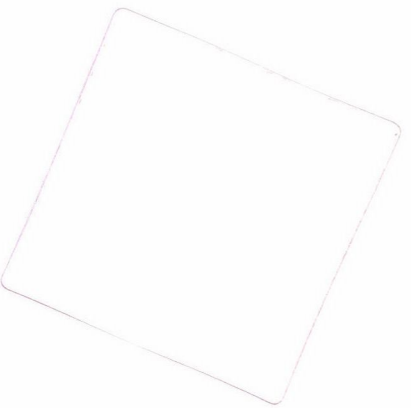

Acta Agronomica Hungarica, 53(4), pp.443–459 (2005)

Review

POTASSIUM UPTAKE BY HIGHER PLANTS: FROM FIELD APPLICATION TO MEMBRANE TRANSPORT

S. KANT[1], P. KANT[1] and U. KAFKAFI[2]

[1]DEPARTMENT OF DRYLAND BIOTECHNOLOGY, J. BLAUSTEIN INSTITUTE FOR DESERT RESEARCH, BEN-GURION UNIVERSITY OF THE NEGEV, SEDE-BOQER CAMPUS, ISRAEL
[2]INSTITUTE OF PLANT SCIENCES AND GENETICS IN AGRICULTURE, FACULTY OF AGRICULTURAL, FOOD AND ENVIRONMENTAL QUALITY SCIENCES, THE HEBREW UNIVERSITY OF JERUSALEM, REHOVOT, ISRAEL

Received: 27 September, 2005; accepted: 7 November, 2005

Potassium (K^+) is a crucial nutrient element for higher plants and plays vital roles in several cellular processes including turgor regulation, stomatal movement, protein synthesis and charge balance. The requirement of K^+ for plant growth changes with the developmental stages and its uptake pattern varies among crop plants. Most annual crop plants take up a large proportion of their K^+ requirement in the initial vegetative growth stage. A deficiency of K^+ during this period may make the plant susceptible to various stresses. Therefore, the timely application of K^+ to the plant rhizosphere is an important factor for achieving better plant growth and yield. Plants take up K^+ by active and passive transport. Electrophysiological and molecular studies done during the last two decades have characterized the active K^+ uptake mechanisms (high and low affinity K^+ uptake systems) and have identified the genes involved in these mechanisms. The knowledge of K^+ uptake during the plant life cycle and of the activation of the K^+ uptake system by the presence of a certain concentration of K^+ in the soil solution would certainly help in planning the rate and time of K^+ application. Therefore, the work done on the pattern of K^+ uptake during plant growth and the mechanism involved in its uptake is reviewed here.

Key words: potassium, uptake, plant growth, mechanism, high affinity, low affinity

Introduction

Potassium (K^+) is one of the most abundant cations in plant cells and is absorbed in large quantities by roots. Plants contain 1–6% of K^+ in the dry matter (Raven et al., 1976; Leigh and Wyn-Jones, 1984). K^+ does not become toxic to most plant species even in the concentration range of 50–100 mM in the cytoplasm (Sheahan et al., 1993). K^+ plays several indispensable roles in normal plant growth, such as turgor maintenance, osmoregulation, stomatal movement, enzyme activation, protein synthesis, charge balance and tropism (Kochian and Lucas, 1988; Schachtman and Schroeder, 1994; Marschner, 1995). The timely, optimum supply of K^+ is helpful in developing plant resistance to various stresses such as drought, salinity, cold damage and lodging (Kant and Kafkafi, 2001a).

K^+ uptake by roots is largely determined by the plant growth rate (Pitman, 1972). The requirement of K^+ changes with the physiological stages of the plants and varies for annuals, biennials, perennials and fruit trees. For optimum crop

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production, it is desirable to know when the crop has its greatest and least demand for nutrients (Kant and Kafkafi, 2001b). Most terrestrial plants take up K^+ directly from the soil solution and are able to sustain growth at widely varying external K^+ concentrations ranging from around 10 μM to 10 mM (Maathuis and Sanders, 1994). The ability to extract K^+ from very low concentrations in the soil solution suggests that the root membranes are able to specifically select K^+ from a solution that contains other cations in much higher concentrations. The mechanisms of K^+ uptake and specific ion channels through which K^+ is selectively taken up are discussed here as well. Numerous investigations since the middle of the last century aimed at understanding the mechanisms of K^+ uptake and its transport in the xylem and phloem. In this review the rate of K^+ uptake during plant growth, its distribution within the plant and K^+ uptake mechanisms are presented.

K^+ uptake pattern in plants

K^+ uptake by roots mainly depends on the growth rate of the plant (Pitman, 1972). Once the older leaves of the plant have reached their characteristic K^+ concentrations, net K^+ flux into the roots only satisfies the amounts needed for the new growth of roots and shoot. The requirement of K^+ to achieve proper plant growth changes with the developmental stages. K^+ uptake usually precedes dry matter production and the uptake of nitrogen (N) and phosphorus (P) (Fig. 1a). At the time of 50% dry matter accumulation in corn plants, 68, 56 and 95% of the maximum N, P and K^+ had been absorbed, respectively (Welch and Flannery, 1985).

At the time of germination the remobilization of nutrients occurs within the seed. After germination ions are translocated through the xylem or phloem towards growing shoots or roots. Seeds of most grain crops contain about 0.4 to 1.0% K^+. This quantity is sufficient for germination and early establishment but not sufficient to maintain further growth (Van-Slyke, 1932). Therefore, the roots have to take up K^+ quickly to sustain the life cycle of the plant. In annual plants, maximum K^+ accumulation occurs during vegetative growth. The K^+ accumulated during the vegetative period is used later for translocation to newly developed tissues, fruits and storage organs (Lawton and Cook, 1954). The K^+ uptake rate in cereal crops starts rising in the early stages of growth when all or most of the leaves are still expanding, with the highest rate during the end of tillering and the start of flowering. The K^+ in the ears comes via translocation from vegetative parts and the K^+ concentration in the leaves starts to decline as the reproductive stage progresses (Fig. 1b; Karlen et al., 1988). The maximum accumulation of K^+ in the aboveground parts of wheat has been found near to the flowering stage. K^+ removal from the soil by the wheat crop may range from about 40 to >500 kg ha^{-1} depending on the production level (Beaton and Sekhon, 1985; Kafkafi and Halevy, 1974). The K^+ requirement of the corn crop during the vegetative period is so high that 59% of the total K^+ uptake occurred during the 21 days between the knee-high and tassel stages (Jordan et al., 1950). Similarly, Hanway (1962) reported that 38% of the total K^+ uptake occurred in

corn during the period 38 to 52 days after emergence. The average daily rate of K^+ uptake during this period ranged from 2.31 to 10.74 kg ha^{-1} (Welch and Flannery, 1985). The rate of K^+ uptake in maize was highest when 50% growth was achieved (Fig. 1c). Thereafter, with the onset of the reproductive phase, the translocation of K^+ started from the leaves and stalks to the developing ears (Fig. 1b).

During blooming or anthesis a further redistribution of inorganic elements occurs in most annual plants. At the early stages of fruit or seed setting there may be a marked increase in mineral absorption by the roots as well as an accelerated metabolism in younger tissues of the plants. A substantial gain in K^+ occurs in cotton plants during boll formation. The uptake of N and P followed that of dry matter production, whereas the K^+ uptake was rapid, reaching a maximum at the time of boll opening (Halevy, 1976). Mullins and Burmester (1990) observed that the maximum accumulation of K^+ in cotton occurred near the start of flowering and uptake rates of 2.2 to 3.2 kg K^+ ha^{-1} day^{-1} were observed during the period 63 to 98 days after planting. Similarly, Hanway (1962) and Gething (1990) observed that most of the total K^+ in aboveground maize plants had been absorbed at the start of the reproductive stage (Fig. 1c). The leaves and stalks contain considerably less K^+ at harvest than at flowering in corn (Fig 1b). Most of the K^+ loss from the leaves and stalk is due to its translocation to the grains. In rice plants 75% of the total K^+ requirement is taken up before the booting stage and the remaining K^+ uptake is completed before grain formation begins (Fig. 1d; De Datta and Mikkelsen, 1985). Similar to grain crops, oilseed crops, e.g. rape, sunflower (Fig. 1e) and flax (Bailey and Soper, 1985), vegetables, e.g. tomato (Lingle and Lorenz, 1969), and forage legumes, e.g. alfalfa and red clover (Lanyon and Smith, 1985), also showed an incremental uptake of K^+ until flowering, whereafter it declined towards maturity. The decline in the absorption of soil K^+ during ripening is mainly due to the decreasing carbohydrate supply to the roots (Lingle and Lorenz, 1969). In contrast to annuals, in biennial crops such as sugarcane and cassava the K^+ uptake is very slow at early stages of growth and keeps on increasing until harvest (Fig. 1f; Filho, 1985; Howler, 1985), since these plants do not go through the reproductive stage before harvest.

K^+ uptake by plants requires energy produced by root respiration, whereas the transfer of K^+ from plant roots to the soil is a simple diffusion process from dead plant parts. In soybean, K^+ loss by fallen leaves and petioles starts 50 days after germination; this loss was rapid during seed filling with complete senescence at maturity (Fig. 1g; Hanway and Johnson, 1985). However, loss of K^+ might also be due to excretion from the roots to the soil (Lawton and Cook, 1954). Mineral analysis at different growth stages of wheat showed that K^+ decreased shortly after heading, and transfer from shoots to roots and then to the soil was also observed (Halevy, 1976). A bale (170 kg) of cotton requires around 52 kg K_2O (43 kg K). The lint and seed remove only about 11 kg K_2O ha^{-1}. Most of the K^+ is in the burs, stalks and leaves, and might return to the soil at harvest (Mullins and Burmester, 1991).

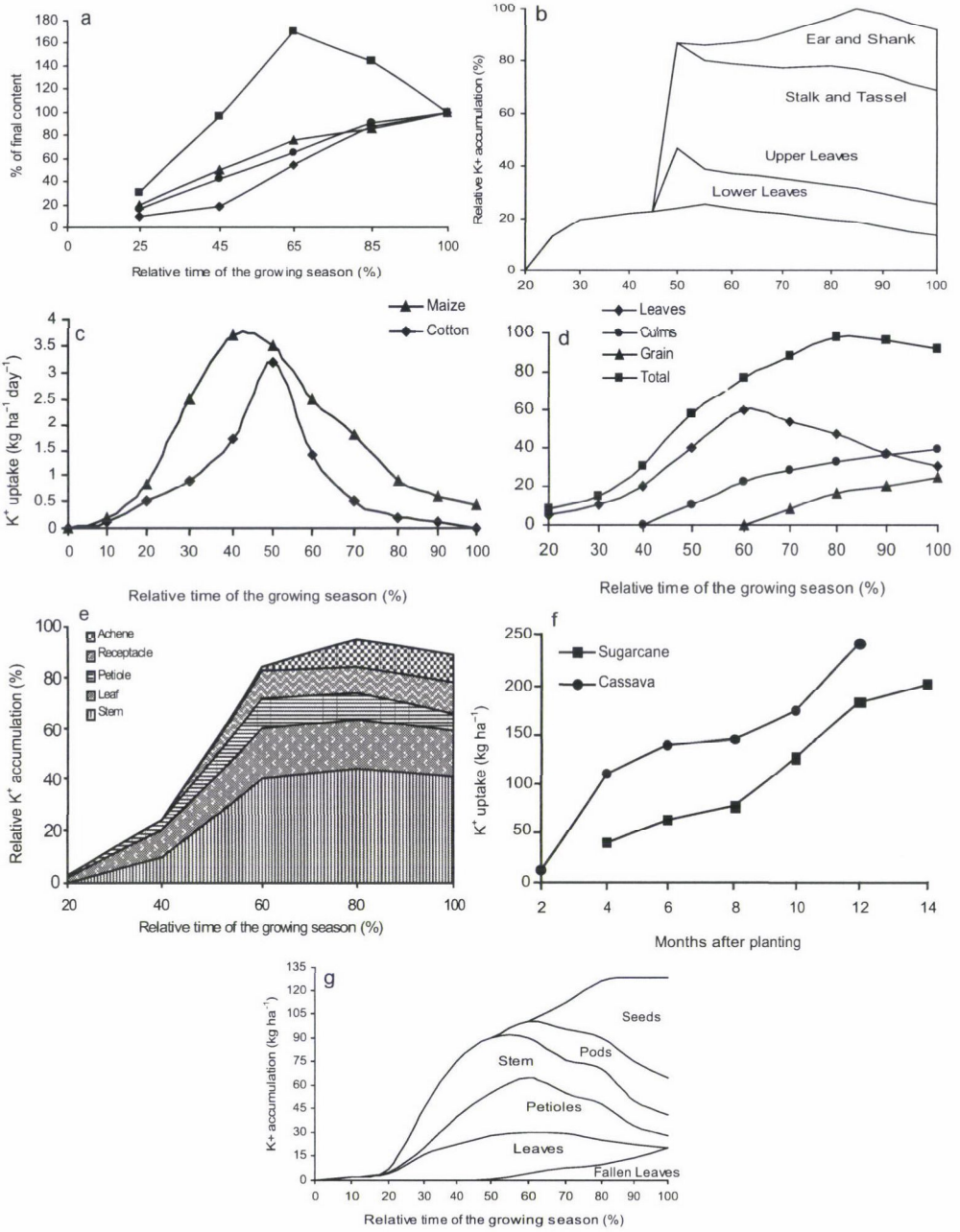

Fig. 1. Total uptake and/or distribution of K^+ with respect to the relative time of growth period (%) in different field crops. (Sources: a: wheat, Russell and Clarkson, 1971; b: corn, Karlen et al., 1988; c: maize, Gething, 1990, and cotton, Mullins and Burmester, 1990; d: rice, De Datta and Mikkelsen, 1985; e: sunflower, Bailey and Soper, 1985; f: sugarcane, Filho, 1985, and cassava, Howler, 1985; g: soybean, Sallam et al., 1985)

K^+ distribution within plants

Once K^+ enters the plant root, it must be translocated to various organs. K^+ is known to be very mobile in plants, moving upward and downward in the xylem or phloem (Fig. 2) in the direction of meristematic tissues (Ben-Zioni et al., 1971; Kirkby and Knight, 1977). A high rate of translocation occurs in the xylem because of the rapid rate at which K^+ is selectively secreted into the xylem vessels. Retranslocation of K^+ from shoot to roots via the phloem also occurs when a substantial amount of nutrients is supplied to the roots. K^+ retranslocation is required to meet the demand for the growth of apical root zones and to balance the fluctuations of K^+ that occur in the external rooting medium (Marschner et al., 1997). Among all the cation species, K^+ is present at maximum concentration in phloem sap (Hocking, 1980), where it may reach concentrations of 100 mM or more. This indicates that K^+ is selectively absorbed by the sieve tubes and can be easily translocated from the upper plant parts to the basal plant organs and roots. The up and down movement of K^+ can be better understood by the experiment of Pitman (1972); barley seedlings were raised in solution containing both K^+ and Na^+. Older leaves accumulated more Na^+ relative to K^+ than younger leaves. The level of K^+ in the leaf cells supposedly maintained active transport from the free space of leaves. The leaf as a whole receives ions from the xylem and can export K^+ (preferentially to Na^+) in the phloem mainly to younger leaves and fruits and also to the roots. The balance between these processes will determine the level of ions in the leaves.

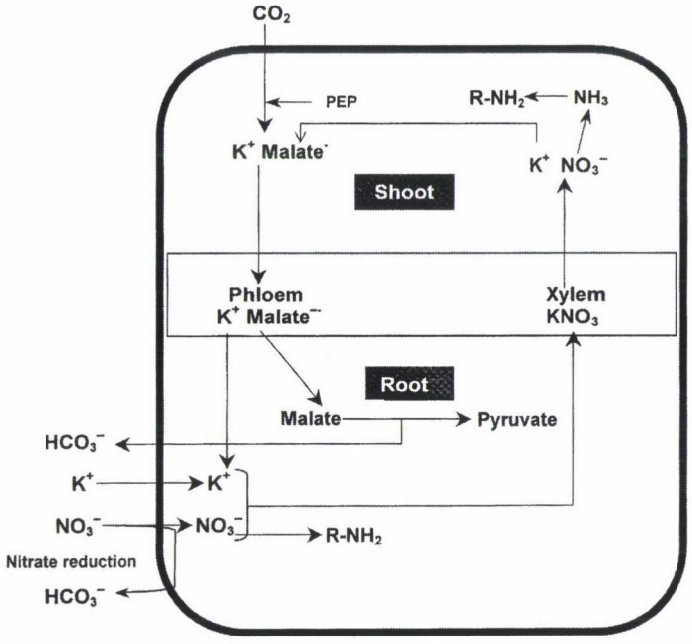

Fig. 2. Model of K^+ mobility between roots and shoot, along with the nitrate and malate circulation (Based on Ben-Zioni et al., 1971; Kirkby and Knight, 1977)

The level of K^+ in the shoots can be regulated either by controlling entry into the shoot or by the retranslocation of K^+ back from the shoot to the root. The movement of K^+ from the leaves to developing sinks by phloem transport is found in all plants (Mengel and Kirkby, 1987) and has been demonstrated in potato (Dijkshoorn, 1972), apple (Hansen, 1980), prunes and plum (Lindner and Benson, 1954). The translocation and redistribution of K^+ occurs from the older plant parts to newly developing parts. During vegetative growth maximum K^+ transport was from stems to branches and in reproductive growth it was higher from stems to pods in soybean plants (Hanway and Johnson, 1985; Sallam et al., 1985). During seed formation the translocation of K^+ to seeds comes from the stem, leaves and roots. The translocation of K^+ from leaves to developing fruits is also noticeable in fruit trees, e.g. peach (Fig. 3), where the leaf K^+ steadily decreased in fruiting trees after flowering, while non-bearing trees exhibited a constant leaf K^+ throughout the growing season (McClung and Lott, 1956).

K^+ interaction with other ions

Most plant species absorb K^+ at a higher rate as compared with that of other cations. Therefore, K^+ is a stronger competitor for uptake with other cations. This cation competition becomes especially evident when plants are grown at sub-optimal levels of K^+ concentration, where the uptake of other cations considerably increased, as shown by Mengel and Kirkby (1980). When the supply of K^+ to barley plants grown in nutrient solution was stopped for one week, this interruption resulted in a sudden increase in the uptake of Ca^{2+}, Mg^{2+} and Na^+, although the total mole charge of cations hardly changed (Table 1).

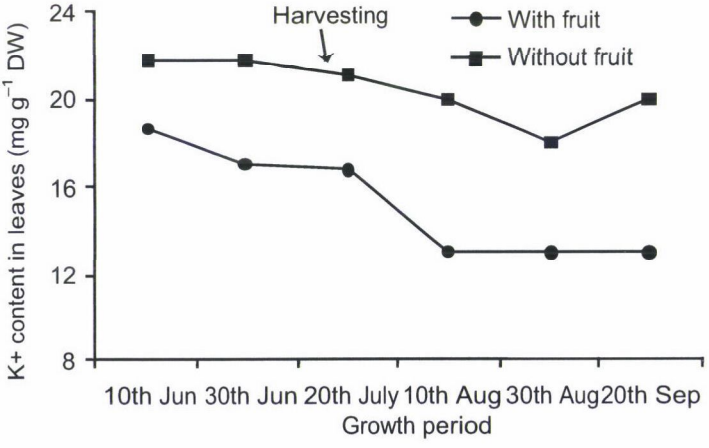

Fig. 3. Effect of fruit bearing on K^+ content of leaves in peach (Source: McClung and Lott, 1956)

Table 1
Effect of K^+ supply on the cation contents (meq/100 g dry matter) in barley

	Shoots		Roots	
	Control	Interruption	Control	Interruption
K^+	170	52	157	28
Ca^{2+}	24	66	9	12
Mg^{2+}	54	121	36	74
Na^+	Traces	12	3	78
Total	248	251	205	192

Interruption means withholding of K^+ supply for one week at the tillering stage (Source: Mengel and Kirkby, 1980)

The presence of ammonium (NH_4^+) near the root suppresses K^+ uptake, while plants supplied with nitrate (NO_3^-) tend to accumulate more K^+ (Kafkafi et al., 1971; Lips et al., 1990). NH_4^+ fertilization prior to K^+ application leads to the fixation of K^+ in non-exchangeable soil sites, causing lower availability of K^+ (Barker et al., 1967). Shukla and Mukhi (1979) reported antagonism between K^+ and both calcium (Ca^{2+}) and magnesium (Mg^{2+}). A deficiency of Ca^{2+} and Mg^{2+} occurs due to competition with K^+ in acidic and high exchangeable K^+ soils (Salmon, 1964). K^+ and Na^+ compete for uptake, but the transport of K^+ is preferential over Na^+. K^+ uptake and its translocation from root to shoot declined drastically under NaCl salinity and was more pronounced when the K^+ level in the growth medium was kept low (Pitman, 1972). Increased K^+ concentration in the nutrient solution reduces Na^+ uptake (Al-Karaki, 2000). Besides Na^+, other cations such as Al^{3+} are also known to interfere negatively with K^+ uptake (Gassmann and Schroeder, 1994). Electrophysiological studies in wheat plants showed the selectivity sequence of monovalent cations to be $Rb^+ > K^+ > Cs^+ > Na^+ > Li^+$ (Tyerman et al., 1997).

The low affinity uptake of K^+ increases with the addition of anions, but the increase depends on the accompanying anion. Phosphate ($H_2PO_4^-$), sulphate (SO_4^{2-}) and particularly nitrate (NO_3^-) show a positive interaction with K^+ (Adriano et al., 1971; Daliparthy et al., 1994). The uptake of K^+ increased linearly in the presence of Cl^-, but with the replacement of Cl^- by $H_2PO_4^-$ or SO_4^{2-} the increase in K^+ uptake was reduced (Fig. 4). This association between the linear component for K^+ uptake and the presence of Cl^- was related to coupling via the saturable Cl^- influx process (Kochian et al., 1985).

Mechanisms of K^+ uptake

The uptake and accumulation of nutrients by plants involve several biochemical and physiological processes occurring both within and outside the plant roots (Mengel and Kirkby, 1980). Plants take up K^+ directly from the external soil solution; the internal K^+ content may rise up to 10^4-fold of the external concentration (Maathuis and Sanders, 1996).

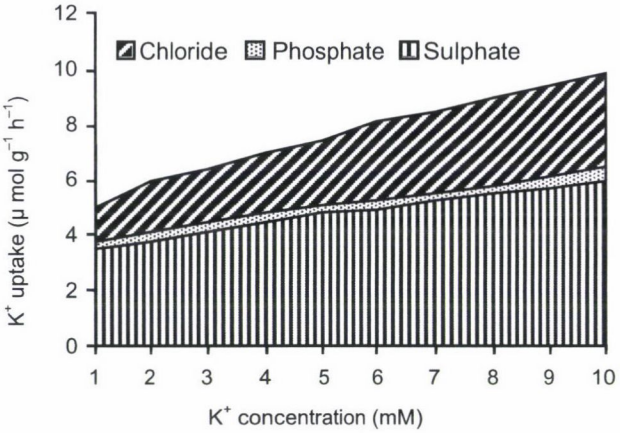

Fig. 4. Uptake of K^+ as influenced by the anions in corn roots (Source: Kochian et al., 1985)

A. Active K^+ uptake by roots

The amount of K^+ intake by plants is directly related to shoot and root growth, root extension and its architecture, which governs the quantity of K^+ accessible to the plant. A dense rooting system can exploit a larger soil volume for K^+ than can a poor one (Mengel and Kirkby, 1980). The root tips of older plants are less capable of absorbing K^+ than those of younger plants (Vincent et al., 1979). Plant species and even cultivars of the same plant species may differ in their capability of exploiting soil K^+. A considerable difference in the K^+ exploiting capability exists between grasses and legumes. Grasses are superior to legumes in K^+ extraction, and when grown together grasses successfully compete with legumes for soil K^+. In soils with low available K^+ the legumes suffer from K^+ deficiency, whereas the grasses grow vigorously (Blaser and Brady, 1950). The K^+ concentration in legume dry matter is almost three times higher than that of grasses; therefore, in soils having low K^+ amounts legumes will show early K^+ deficiency (Kafkafi et al., 1977).

Most plant roots have the ability to take large amounts of K^+ from soil solutions that usually contain low levels of soluble K^+. The roots have evolved an uptake mechanism that operates at low external K^+ with high efficiency (Epstein, 1973). When the rate of K^+ uptake was examined over a wide range of external K^+ concentrations, Epstein et al. (1963) and Epstein (1972) suggested a dual pattern of low affinity and high affinity K^+ uptake by barley roots. The high affinity system has affinity for K^+ in the range of ~4 to 40 μM, with saturation at 300 μM, while the low affinity system has an average affinity range of ~1 to 20 mM (Epstein et al., 1963; Kochian and Lucas, 1988; Kim et al., 1998). Because plants encounter a wide range of soil conditions, these two high and low affinity K^+ uptake mechanisms (Fig. 5) were assumed to exist in parallel and to work simultaneously in the plasma membrane (Kochian and Lucas, 1988). This biphasic K^+ uptake model was used to explain how plant cells maintain a

cytosolic K^+ level several times higher than that at the root surface. The introduction of modern electrophysiology and patch-clamping techniques has made it possible to study the K^+ absorption mechanism at the molecular level. Work in the past decade has confirmed the involvement of ion channels in low affinity K^+ uptake; their role will be discussed later in this review.

i) High affinity K^+ uptake

The high affinity K^+ uptake system is assume to be mediated by a carrier that functions when the K^+ concentration is in the micromolar range (Fu and Luan, 1998). By coupling the K^+ and other cations it moves K^+ into the cytosol against the electrochemical gradient (Kochian et al., 1993; Maathuis and Sanders, 1993). This mechanism must be energized despite the presence of a highly negative membrane potential. Maathuis and Sanders (1994) studied the mechanism of energy transfer using patch clamp electrophysiology. They used root protoplasts of *Arabidopsis thaliana* to characterize membrane currents resulting from the application of micromolar K^+. In their study, K^+-dependent membrane currents were independent of ATP and were reversed at potentials that implied H^+-coupled K^+ transport at a ratio of one to one. This H^+-K^+ coupled symport mechanism was competent in driving K^+ accumulation to equilibrium ratios in excess of 10^6-fold. Several different mechanisms for K^+ uptake have been described at the plasma membrane, e.g. primary pumping of K^+ through ATP hydrolysis (Stein, 1990); symport with Na^+, in which the downward movement of Na^+ provides the driving force, as in chlorophytic algae (Smith and Walker, 1989; Walker and Sanders, 1991); symport with H^+, as in *Neurospora crassa* (Rodriguez-Navarro et al., 1986). The existence of one of these systems forms the basis for K^+ energisation in the roots, where an ATP-fuelled K^+-motive pump or K^+/H^+ exchange pump has been the subject of particular speculation (Kochian et al., 1989).

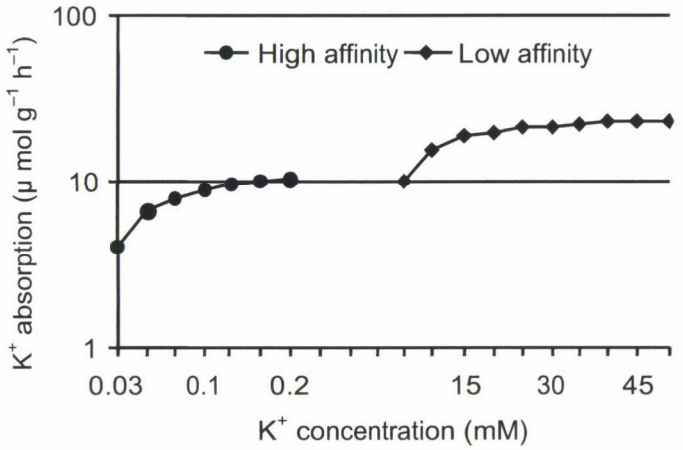

Fig. 5. High and low affinity uptake of K^+ by barley roots (Note: the concentration scale has been changed after 0.2 mM K^+; Source: Kochian and Lucas, 1988)

The plant K^+ status may have a profound influence on up- or down-regulating the activity of K^+ transporters. K^+ starvation in the roots enhances high affinity transport uptake while not affecting low affinity transport in corn (Kochian and Lucas, 1983), ryegrass and barley (Glass and Dunlop, 1978). However, Benlloch et al. (1989) observed that uptake in both the high and low affinity range increased after starving sunflower plants of K^+. Likewise, kinetic studies on K^+ uptake in barley demonstrated the down-regulation of high affinity uptake after growth in 1 mM external K^+ concentration (Fernando et al., 1990). During the last decade several genes encoding high affinity K^+ uptake have been identified in higher plants, for example *HKT1* (high-affinity K^+ transporter) in wheat roots (Schachtman and Schroeder, 1994); *HvHAK1* (high-affinity K^+ transporter) in barley (Santa-Maria et al., 1997); *AtKUP1-4* (K^+ uptake transporters) and *AtKT1-2* (K^+/Na^+ transporter) in *Arabidopsis thaliana* (Kim et al., 1998; Quintero and Blatt, 1997). Molecular studies showed that the *HKT1* gene was expressed in root cortical cells and in cells adjacent to the vascular tissue in leaves of wheat seedlings (Schachtman and Schroeder, 1994). The K^+ starvation of barley and wheat roots resulted in a rapid, strong up-regulation of the *HKT1* mRNA level. Re-supply of 1 mM K^+ was sufficient to strongly reduce *HKT1* transcript levels. Expression levels of *HKT1* were correlated with an increase in high affinity K^+ uptake and preceded any detectable changes in shoot or root K^+ (Wang et al., 1998).

ii) Low affinity K^+ uptake

The low affinity system functions as a passive transporter responsible for K^+ uptake (Rodriguez-Navarro, 2000). This pathway comprises the dominant mode of K^+ uptake at external K^+ concentrations above 1 mM. The low affinity K^+ uptake system is activated in response to the negative membrane potential generated by the H^+ pump and is mediated by voltage-gated inward K^+ channels. It is often non-saturable as a function of K^+ concentration and is specifically inhibited by K^+ channel blockers, and does not appear to be energized (Kochian and Lucas, 1988; Maathuis and Sanders, 1993). In order to function as a low affinity K^+ uptake pathway, channels should be open when the electrochemical potential for K^+ is inward and closed if the opposite occurs, when the cytoplasmic K^+ concentration is around 100 mM (Leigh and Wyn-Jones, 1984). This would ensure the opening of the channel if the external K^+ is in the millimolar range and closure in the micromolar range.

Ion channels

K^+ channels control low affinity K^+ uptake, which represents a major component of K^+ uptake in plants. The ion channels are integral membrane proteins that play an indispensable role in solute transport across the membrane (Maathuis and Sanders, 1995). These ion channels have two unique properties, one of which is selectivity (ion recognition). The cation selectivity for different

K^+ channels varies widely, from highly selective for K^+ to a virtual absence of cation discrimination (Maathuis et al., 1997). The other property is the ability to reside in opening and closing conformational switching during ion permeation (Schachtman et al., 1991). For the movement of K^+ across the membrane, K^+ channels act in two ways. One opens under more hyperpolarizing conditions (at negative membrane potentials in the range –50 mV to –150 mV) and has been found to be capable of K^+ uptake (Schroeder et al., 1994; Very and Sentenac, 2002). These channels are therefore termed inward-rectifying K^+ channels (K^+_{in}). The second type opens under depolarizing conditions (at a positive voltage, more than the K^+ equilibrium potential) and carries an outward K^+ movement. These are referred to as outward-rectifying channels (K^+_{out}) (Tester, 1990; Very and Sentenac, 2002). The concentration of K^+ can affect the opening and closing of K^+ channels by altering the membrane voltage. In stomata guard cells relatively little change in activation was observed when the external K^+ changed from 10 to 100 mM. However, in the range from 0 to 100 mM K^+, the activation voltage does respond to alterations in external K^+ (Schroeder and Fang, 1991).

Several genes involved in K^+ transport (K^+_{in} and K^+_{out}) have been identified, encoding K^+ channels in *Arabidopsis,* e.g. *KAT1* (Anderson et al., 1992), *ATK1* (Sentenac et al., 1992), *ATK2* (Cao et al., 1995) and *SKOR* (Gaymard et al., 1998), and in maize, *KZM1* (Philippar et al., 2003). The expression of K^+_{in} and K^+_{out} channel genes is highly tissue-specific. The activation of K^+_{in} channel has been described in various plant tissues: *KAT1* and *KAT2* are expressed in guard cells (Nakamura et al., 1995; Bruggemann et al., 1999) and *SPIK* in pollen grain protoplasts (Fan et al., 2001), while *AKT1* is predominantly expressed in the epidermis, endodermis and cortex of roots (Sentenac et al., 1992; Ivashikina et al., 2001). Similarly, K^+_{out} channels have been reported to be tissue-specific, e.g. *SKOR* in xylem vessels of the roots (Gaymard et al., 1998) and *GORK* in guard cells, vasculature and roots (Ache et al., 2000; Ivashikina et al., 2001). Besides their major role in K^+ uptake and transport via low affinity K^+ uptake, K^+ channels are also involved in several physiological processes in plants. These are the K^+ uptake in plant cells during their growth and expansion (Schroeder et al., 1994), xylem loading and unloading (Wegner and Raschke, 1994), membrane voltage regulation (Ward et al., 1995), and solute movement to control stomatal opening, osmoregulation and leaf movements (Maathuis et al., 1997). In addition to K^+_{in} and K^+_{out} channels, another weakly selective cation channel permeable to both K^+ and Na^+ has been reported to be active in the mesophyll of *Arabidopsis* (Spalding et al., 1992). This channel seemed to allow both the influx and efflux of ions (Very and Sentenac, 2002). The cation channel genes identified in plants and animals have been regrouped into six families: Shaker, KCNK, Kir, cyclic-nucleotide-gated channels (CNGCs), glutamate receptors (GLRs) and two-pore putative calcium channels (TPCs). The Shaker family is the most thoroughly studied family for cation transport in plants (Very and Sentenac, 2002). To date, nine K^+ channel

genes (*KAT1*, *KAT2*, *KAT3*, *AKT1*, *AKT2*, *AKT5*, *AKT6*, *SKOR* and *GORK*) belonging to the Shaker family have been identified (Maser et al., 2001).

The existence of high and low affinity K^+ uptake systems ensures large flexibility for plants to acquire K^+ under conditions where the soil solution K^+ may vary between micromolar and millimolar levels. Since plants encounter a wide range of soil conditions, it is likely that multiple high and low affinity uptake mechanisms exist.

B. Passive K^+ uptake by roots

The uptake of K^+ is directly proportional to its amount and concentration in the soil solution (Barley, 1970). K^+ moves in plants along with the transpiration stream. With an increase in transpiration, the long-distance transport of K^+ via xylem vessels generally increases (Marschner, 1995). However, the distribution of K^+ in xylem sap may vary with the plant organ, especially in fast growing plants. For example, in barley the K^+ concentration varies from the base of the leaf blade to the tip, from 18.0 to 80.0 mM (Wolf et al., 1990). The total K^+ uptake by plant roots and the amount of K^+ in the shoot increases with plant size, especially in cereals, where the K^+ level increases rapidly during the vegetative growth period (Russell and Clarkson, 1971; De Datta and Mikkelsen, 1985).

Conclusions and future prospects

Summing up, the available information reveals that the uptake of K^+ in annual crops reaches its peak before flowering, with a decline towards maturity, while biennial and perennial plants take up K^+ throughout the growing season. This suggests that there is a short period during the growing season of annuals when K^+ deficiency could be corrected. It seems prudent in the case of cereals to ensure adequate K^+ presence in the soil before sowing or during the early growth stage rather than planning to make corrective application at a later stage. An insufficient K^+ supply adversely affects the reproductive organs, thereby reducing the crop quality and the quantity of economic yield. Electrophysiological and molecular studies have identified high and low affinity K^+ uptake systems and have isolated several K^+ channel genes in different plant tissues. A rich knowledge on K^+ uptake mechanisms is available, but the application of this knowledge to farmers' fields is of practical importance. There are many puzzles that must be resolved to achieve a comprehensive understanding of K^+ nutrition, for example: the mechanisms of the K^+/H^+ antiporter and K^+ permeation, which have been little studied, how and when the genes for the K^+ channel are activated during the plant growth cycle, how the K^+ uptake process is affected by soil pH, and how other nutrient ions interact with K^+ channels during its uptake and transport.

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ISSN 0238-0161

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